A study of herbaceous vegetation in Chequamegon – Nicolet National Forest: Relationship of earthworms, white-tailed deer browsing and *Carex pensylvanica Lam*

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Dedication

To Susan and Chris for awakening a forgotten wish. To my mother and father for the love of learning. To all who supported and helped. To all my friends and my father who passed during this: Dad, Aunt Esther; John Steinnes; Earl and Kathy Hoagland. Winonah and Maajii, my loyal companions the first year

Abstract

Invasive earthworms alter multiple forest components. By accelerating litter decomposition, they alter nutrient flows, soil composition and vegetative communities. White -tailed Deer (*Odocoileus virginianus)* are known to alter vegetative communities by selective browsing; severity varies with population density and affects plant community population and composition. Both factors are associated with reduced vegetative community richness and dominance by graminoids. In this study, 101 randomly selected Northern Mesic Hardwood Sugar Maple sites in the Chequamegon – Nicolet National Forest were sampled for vegetation, earthworm occurrence and browsing intensity. Over three years, eighty-two percent of sites were positive for earthworms; in two non-drought years, ninety percent of sites were positive. Non-metric Multi-dimensional Scaling (NMDS) and Multiple Response Permutation Procedure (MRPP) found divergent communities; a *Carex pensylvanica Lam* dominated community associated with earthworm invasion and strongly linked to *Lumbricus rubellus* presence, and remaining *Acer saccharum* seedling stands associated with reduced earthworm impacts. Additionally *Carex pensylvanica* was strongly linked to *Lumbricus rubellus* presence by Indicator Species Analysis. Lumbricus rubellus invaded sites had both reduced species richness and vegetative cover. White-tailed deer (*Odocoileus virginianus*) browsing was found to be heavy and extensive throughout both forests, impacting *Acer saccharum* regeneration and further driving graminoid dominance. The results indicate earthworm invasion is geographically extensive and a principal driver of *Carex pensylvanica* understory dominance and reduced *Acer saccharum* regeneration.

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Introduction

Forests are dynamic systems, with interactions at scales ranging from microbial to climatic variation. Forest spatial heterogeneity is driven by *disturbance*, or change, caused by both human and natural activity, extending from cutting individual trees and clear-cuts (human) to microbial invasion (Liebhold et al. 1995) and regional events, such as hurricanes or ice storms (Pickett and White 1985, Irland 2000). Natural processes include insect outbreaks, wind and fire, the latter two being primary drivers of forest spatial heterogeneity (Frelich 2002). Species migration, introduction and extinction rates exist within this larger framework, limited by dispersal ability, environmental variation and competitive interactions (Husband and Barret 1996). Species introduction by humans and subsequent invasion fits within the disturbance dynamic framework (Liebhold et al. 1995). Successful invaders, regardless of taxa, possess traits assisting their exploitation of new environments (Strayer et al. 2006, Matzek 2012). Invasion success is also dependent on the introduction landscape, with previously disturbed landscapes more vulnerable to invasion success (Sher and Hyatt 1999, Harper et.al. 2005, MacDougal and Turkington 2005). Plant invasive species also adapt to the new environment via invasive release, or freedom from insects, browsing, or pathogens that occur in their native environment (Bais et al. 2003, Pisula and Meiners 2010). However, this response is not uniform across species (Chun et al. 2010).

Invasive earthworms and White-tailed deer browsing

Invasive earthworms are now recognized as a driver of vegetation community change in North America (Frelich et al. 2006, Bohlen et al. 2004, Hendrix 1995). Native earthworms were minimally present north of the last (Wisconsin) glacial advance and none of those present in this study are native to the continent (Hendrix 1995). Invasive earthworms cause soil profile changes, increased soil bulk density, and lower nutrient availability (Hale et al. 2005b, 2008), reduced wildflower presence (Holdsworth et al. 2007), increased rates of litter decomposition, and increased bare surface soil presence (Suarez et al. 2006, Gundale et al. 2005). Additional consequences are facilitation of invasive plant taxa and greater graminoid dominance (Frelich et al. 2006, Eisenhauer et al. 2009). Holdsworth et al. (2007b) found greater sedge cover and lower maple seedling recruitment in Chequamegon National Forest; borrowing a term from previous work, they are "ecosystem engineers," restructuring the forest (Holdsworth et al. 2007a).

Of the eight invasive earthworm species commonly found in forests of the western Great Lakes Region, two—*Lumbricus terrestris* (night crawler) and *L. rubellus* (leaf worm), are thought to cause most of the change in environment that leads to plant community change (Hale et al 2005b, Hale et al. 2006, Holdsworth et al 2007b, 2008). *Lumbricus terrestris* is normally a late stage invader their feeding preference is fresh litter (Loss et al. 2013). They dominate after previous species have altered the litter and soil profile so fresh vegetative litter is reachable from their middens (Hendrix 1995). As their population density increases, most or all of a year's litter will be consumed and the signature "bare soil" with middens may dominate the understory (Frelich et al. 2006; Suarez et al. 2006). *Lumbricus rubellus* is described as an "epi-endogeic" species, living in both the litter and upper mineral soil layer. They alter the forest floor, directly consuming older litter in the F and H layers, altering the soil profile, and are subsequently associated with changes in plant community composition (Hale 2005; Frelich et.al. 2006).

Herbivory - in this case browsing by white-tailed deer (*Odocoileus virginianus)* - can be viewed as a "chronic" disturbance, persistent across space and time while interacting with other agents (Wisdom et al. 2006). Selective browsing alters community structure, and can shift forest landscapes to domination by resistant survivors (Coté et al. 2004). Previous studies from the same region as this study link deer herbivory to increased graminoid cover, decreased plant species richness and reduced tree species regeneration (Rooney and Waller 2003, Waller and Alverson 1997, Rooney 2008). Powers and Nagel (2009) found differing combinations of even age (intense) stand management, heavy deer browsing and high earthworm populations led to the greatest *Carex pensylvanica* cover; two factor combinations of "forest management, high deer density and-or dense epigeic earthworm populations" were associated with higher *Carex pensylvanica* cover generally. This study examines vegetative community change by comparing stands with differing impacts of earthworm invasion and white-tailed deer herbivory in 101 stands of similar composition and disturbance history in the Chequamegon-Nicolet National Forest.

Study Hypotheses

This study builds upon previous work in the Chequamegon-Nicolet National Forest and tests three hypotheses based on the findings of past examinations of earthworm impacts and herbivory on sugar maple-dominated forests:

H1: 80% or more of sites will have earthworms present and/or are in an impacted state.

H2: Graminoid abundance will be associated with *Lumbricus rubellus* and *Lumbricus terrestris* presence

H3: Browsing should be extensive across the landscape and variation in browsing intensity should be related to graminoid occurrence. Occurrence of palatable or preferred species and abundance of tree regeneration attaining heights of one meter or more should be reduced.

Methods

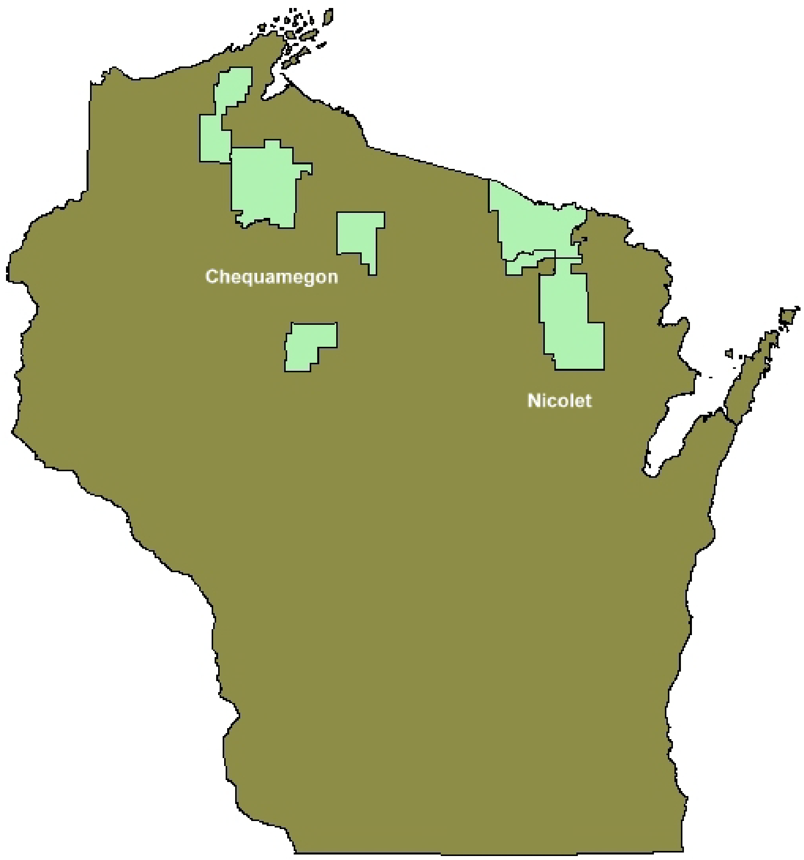
Study area

The study was conducted in Chequamegon-Nicolet National Forest in the State of Wisconsin during the years 2007 and 2009-2010. Chequamegon-Nicolet National Forest consists of two separate landscapes administratively joined:

The Nicolet unit is centered in Northeastern Wisconsin near 45° 40' 47 North Latitude 88° 44' 37 West Longitude. Its northern border is the state boundary with Michigan, and averages about 65 miles north to south and at its widest is about 35 miles wide. It is generally one contiguous landscape with mixed ownership surrounding and within.

The Chequamegon unit is in western North Central Wisconsin and consists of three separate landscapes. The largest is centered at 46° 14' 12 N Latitude 90° 59' 58 W

Longitude. This block runs from about 60 miles north to south; at its widest it is nearly 30 miles. Another block, east of Park Falls, WI, is centered at 45° 52' 24 N Latitude 90° 12' 18 W Longitude and runs about 22 miles north to south and 15 miles east to west. A third block, near Medford, WI is centered at 45° 16' 32 N Latitude 90° 33' 53 W Longitude (Map 1).



Map 1: Chequamegon-Nicolet National Forest

The landscape for both forests is glacial topography with hills, moraines and glacial outwash plains; local relief is low with hills ranging from 10-50 m (USDA 2004). The climate for both landscapes is humid continental – warm summer subtype, but has experienced warming and drought related to global warming in the last few decades (Swanston et al. 2011; Albert 1995). Soils are glacial in origin and dominated by loams;

soils on Nicolet tend to be richer, with the northern area having a silt loess cap. Chequamegon tends also to loamy soils, but with more sand mixture (Albert 1995, http://websoilsurvey.nrcs.usda.gov/. Accessed 6/15/2014).

Field methods

Sampling sites were selected from the Forest Service's previously inventoried Great Lakes Maple-Basswood Mesic Hardwood Forest stands. Stands were identified by U.S. Forest service Stand ID and assigned a random number in Microsoft Excel. A number of sites deemed sufficient for a field visit were selected and mapped by geographic centroid and assigned as locations for navigation. Sample plots were thirty meters by thirty meters square, located in relatively uniform landscapes without breaks such as wetlands or bedrock. Four nested vegetation subplots were located at four central ten meter grid points within the main plot. Two worm sampling points were located on the vertical axis between the vegetation plots at the fifteen meter midline. Earthworms were sampled by litter search, liquid mustard sampling and midden counts (See Earthworm Sampling pp 7). Understory vegetation including shrubs and saplings was measured in each subplot and canopy trees were classified for basal area by species using a ten factor prism from the center of the main plot. Deer browsing intensity on sugar maple saplings between one to five feet tall was estimated by percentage of stems available to deer browse (Frelich and Lorimer 1985). Vegetation within the main plot outside of the subplots was classified by low, medium and high abundance. Soil was sampled with a fifteen by fifteen centimeter square collected from each subplot creating a composite plot sample. Data were recorded on the field data sheet (Appendix A) and Worm Data Sheet (Appendix B). Sites for the day were selected by proximity for a day's travel. If a site was too removed or rugged for overland travel in a reasonable time, it was abandoned and another site was attempted. During foot travel, GPS units often lost signal in the canopy and this might have selected for road proximity. If so, this could lead to a minor bias in the data for more positive worm results and greater deer-browsing effects (Holdsworth 2007b). One hundred-one sites were sampled over three years, equally dispersed across both units of the forest (Appendix C).

Earthworm sampling

Three different types of earthworm data were collected: liquid mustard extraction from the soil, midden counts for *L. terrestris* (the only midden-producing species in this region), and for plots installed in 2009 and 2010, stage of earthworm invasion. Earthworms were extracted from the soil by the widely-used liquid mustard sampling method following Lawrence and Bowers (2002) and Hale et al. (2005a). The mustard is an irritant that causes worms to surface. Liquid mustard extraction involves the following steps:

1. Press a 0.125 m^2 sampling frame (35 cm x 35 cm) into soil to a depth of ca 1 cm.
2. Measure forest floor depth, search leaf litter for worms as leaf litter is collected.
3. Place worms found in tray with alcohol and litter in marked paper bag.
4. Count middens (see details in following paragraph).
5. Shake mustard solution (75 ml of ground yellow mustard in 3 l of water) and pour ~1/3 of it evenly over the area within the sampling frame.

6. Collect worms that surface and place into tray with alcohol until emergence notably slows down (wait about 5 minutes).

7. Pour second ~1/3 of mustard solution and collect worms until emergence notably slows down (wait about 5 minutes).

8. Pour last 1/3 of solution and wait until one minute passes without emergence of worm, at which point the extraction process stops for a given subplot.

9. Move worms from trays to labeled plastic screw-cap vials.

As mentioned above, an additional index of *Lumbricus terrestris* density was obtained by counting middens, which are defined as holes (burrow entrances) 2-6 mm wide, ringed by castings with litter parts (petioles and/or other leaf parts) partially pulled into them. If no middens were found in the two liquid mustard extraction subplots, then two additional subplots were placed within a 3.5 m radius to search for middens.

Earthworm invasion is a progressive phenomenon. Previous studies have documented vegetative community and soil impacts progressing through stages as worm species and litter biomass change (Suarez et al. 2006, Hale 2005a, Holdsworth et al, 2008). While this study was in progress, a preliminary rapid assessment protocol of earthworm invasion with four stages of invasion based on visual clues of forest floor condition was developed by Frelich, which was later published as a five-stage assessment protocol by Loss et al (2013). Frelich's four-stage assessment was used here, and it is essentially the same as the five-stage protocol of Loss et al. (2013), but with stages 4 and 5 merged into stage 4.

Frelich Four Stage Assessment:

Stage 1: Earthworm free, with thick leaf litter, fresh litter on top matted, with layers stuck together.

Stage 2: Epigeic (litter dwelling) earthworm species *Dendrobaena octaedra* present. Leaf litter almost as thick as in category 1, and there is a substantial F layer present. Fresh litter is not matted and stuck together.

Stage 3: Endogeic (soil dwelling) earthworm species including *Aporrectodea* spp., *Octolasion* spp. and *Lumbricus rubellus* (or some combination of them) present, and possibly a few *L. terrestris*. A thin F layer and thin layer of fresh litter is present, and a thick black A horizon has begun to develop.

Stage 4: *Lumbricus terrestris* invasion is complete and the species is present in large numbers, although the species from earlier stages are still present, but at lower abundances. No F layer is present, there is a thick black A horizon, high midden density, and fresh litter is present in fall and spring, but bare mineral soil is common by midsummer.

Understory vegetation data collection

Vegetation was measured by surface coverage in classes or density as described in the following protocol:

1: On the four 2 x 2 m subplots, identify species of plants up to 1 m tall in subplots, including herbaceous plants, tree seedlings and shrubs, and assign each species to one of six cover classes: <1% = Class1;1-5% = Class 2; 5-25% = Class3; 25-50% =Class4; 50-75% = Class 5; 75-100% = Class 6 following Braun-Blanquet.

2: On the four subplots observe and record percent cover and density of tree saplings greater than one meter tall, but no greater than 2.5 centimeters diameter at breast height (dbh). Record percent cover in the same classes as less than one meter vegetation and the following density classes per taxa, 1-5=1; 5-10=2; 10-20=3; 20-30=4.

3: Observe tall shrubs over 1 meter throughout full plot, classify by species and % cover.

During field work, plants occurring on field plots were resolved to the species level unless unidentifiable; nomenclature and symbols follow the Natural Resource Conservation Service Plants Database (NRCS, http://plants.usda.gov) unless none existed (Appendix D). Because sampling occurred in mid to late summer, some plants were not identifiable to species; this was especially true during the 2007 drought. If a plant was unidentifiable, it is listed as "UNIDSP". Certain taxonomic groups were created when identification to genus but not species was possible; "TRISP" for Trillium taxa or "CARUN" for unidentified *Carex* taxa are examples.

Data were recorded and then transferred to an electronic file. One large data file with all the recorded fields was created, data was then separated into main matrices for later use; one was for all year's data, and the second for 2009 – 2010.

Data Analyses

General.

For exploratory analyses, data were summarized by forest unit (Chequamegon or Nicolet) and year (2007, 2009 and 2010). Due to a drought during 2007, two data sets were compiled, including 101 field plots from 2007, 2009 and 2010, and a second for the two non-drought years, 2009 and 2010 (n = 62 plots). All analyses were run for both data sets; however, the detailed ordination and species composition analyses presented below are from the 2009-2010 dataset only. The sample size of 62 plots tested as sufficient for analysis (Appendix G).The ability to relate the plant data to earthworm abundance was better for this data set, since earthworms were less active during the drought-affected 2007 field season. In addition, the protocol for earthworm stage of invasion was available for the 2009-2010 field seasons.

Multi-variate Analyses

Multi-variate analyses including ordination, indicator species analyses, multiple response permutation procedure and the biodiversity measures species richness and evenness were used to characterize the relationships between earthworm presence and plant community composition. Data normality was tested using the Anderson – Darling Normality Test (Otto 2005). *Acer saccharum* area coverage data was used, being a target species and second most common. The key result is the *P-value* for the normality hypothesis: *P* greater than alpha > 0.05 indicating a normal distribution.

Non-metric multi-dimensional scaling (NMDS) was chosen for ordination analyses of the understory plant communities (as opposed to the parametric principal components or factor analysis methods) as the method does not require assumptions for the process, allows different distance measures to be used or the data to have a linear relationship (Kruskal 1964, McCune and Grace 2002). NMDS is a non-parametric method that places samples into a rank order along a gradient, then reduces and maps the higher dimensional data into lower dimensions. The data is visually displayed, with distance among points on a graph symbolizing their similarity – dissimilarity relationships. The goal is to map the data points as close as possible to the data's actual "distance" relationship from each other in the real world. The "stress" is a measure of the difference between the real data and the final configuration at the chosen lower dimension, which is used to determine whether a stable and useful ordination has been obtained (Kruskal 1964; McCune and Grace 2002).

Details of NMDS analyses were as follows:

1: Data for vegetation less than one meter height (mean abundance of the four 2x2 m subplots) was input into a matrix with sample sites as rows and taxa as columns; the ordination output is then samples in "species space", meaning the closer sample sites are to species points, the more dominant the taxa is within the sample site.

2: Data were relativized by row maximum so differences within a plot sample unit was minimized as some species dominated both occurrence frequency and cover (e.g. *Carex pensylvanica*).

3: The data were arcsine square root transformed to reduce data spread. The method is recommended for proportional data and was chosen as *Carex pensylvanica* dominated all other taxa (McCune and Grace 2002; Sokal and Rohlff 1995).

4:   Origination and end points for the NMDS ordination were set by a prior Bray-Curtis ordination; the method first sets endpoints or poles for each species or taxa.

5: Distance Measure used was Relative Sorensen, also known as "Bray-Curtis Distance". The total distance between any pair of plots is the sum of the differences in relative abundance across all taxa, found in prior studies to be a useful measure for non-metric ordinations (McCune and 2002).

6: For NMDS and MRPP, rarer taxa were eliminated to reduce information noise. This was done as many taxa were rare or relative cover percentage was small. The remaining species were the most frequent and highest in cover and retained the necessary information for analysis (Poos and Jackson 2012)

7. PC-ORD 5.33 was used to run the ordinations using the ‘Slow and thorough’ autopilot mode, random starting coordinates, 250 random runs, and 250 real runs. The best ordination, as indicated by final stress, was the 2009-2010 data set, including species that were present in at least 15% of stands (see results).

8. To examine the effects of factors that may be related to plant community structure as revealed by ordination, an environmental matrix was used for overlay analyses on the final ordination. It included the following variables:

- *Lumbricus terrestris* Presence or Absence
- *Lumbricus rubellus* Presence or Absence
- *Dendrobaena octaedra* Present or Absence
- *Lumbricus immature* Presence or Absence
- Invasion stage (Frelich four stage)
- Nonpigmented (endogeic) worm taxa Presence or Absence
- Deer browse class intensity: high, medium or low.

Indicator Species Analysis

Originally described by Dufrene and Legendre 1997; the method uses species as indicators for membership within *a priori* defined groups; species relative frequency and relative abundance and “loyalty to group” is compared within and between groups. The

"Indicator Value" is 100 or "maximum when all individuals of a species are found in a single group of sites and when the species occurs in all sites of that group". Statistical significance is evaluated by a randomization procedure (Dufrene and Legendre 1997). The defined groups were the Frelich Earthworm Effects Categories.

Species Richness Analysis.

Two groups of stands with different plant communities as indicated by ordination analyses were selected based on their location on the final/best ordination obtained, 22 sites each for both *Carex pensylvanica* and an *Acer saccharum* regions. Pc-Ord 5.33 species richness summary and species richness curve functions were used (McCune and Grace 2002) to extract the following measures from each of the 2 groups:

- Mean total cover of all taxa among the sites included
- Mean number of taxa per site
- $E = Evenness \;\; = \frac{H}{ln\,(S)}$
- $H \;=\; Shannon\ diversity\ index \;=\; -\sum_{i=1}^{s} p_i \ln p_i$
- D= *Simpson`s diversity index for infinite population* = $D = 1 - \sum_{i=1}^{s} p_i^2$
- 1st Order Jackknife estimator: $Jack_{1} = S + \frac{r1(n-1)}{n}$ where S=observed number of

species, rl = number of species in only one sample unit and n= number of sample units.

- 2nd Order Jackknife estimator: $Jack_{2} = S + \frac{rl(2n-3)}{n} - \frac{r2(n-2)^2}{n(n-1)}$ where r2 = the number

of species occurring in exactly two sample units.

Group Difference Test: Multiple Response Permutation Procedure

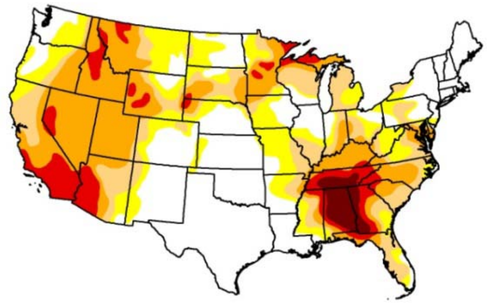
To test if there was a significant difference between the two groups, two groups of data were tested by Multiple Response Permutation Procedure, a nonparametric test for testing the hypothesis of no difference between two groups (Biondini, Bonham & Redente 1985). The same groups used for the Species richness comparison were used in the MRPP test. Data was transformed by arcsine square–root and the distance measure was relative Sorensen. To clarify results, rarer taxa in less than five percent of stands were eliminated leaving 34 for the test. The procedure within Pc-Ord is as follows:

- Calculate distance matrix of responses between sample units.
- Calculate the average distance within groups.
- Calculate delta, the weighted mean group distance (weight refers to number of occurrences) : $delta = \sigma = \sum_{i=1}^{g} C_{i\, x_i}$
- Calculate Probability of delta this size or smaller
- Calculate test statistic T:= $\frac{observed\ \delta - expected\ \delta}{standard\ deviation\ expected\ \delta}$
- Calculate chance corrected within group agreement statistic A= $\frac{1 - observed\ \delta}{expected\ \sigma}$

Results

Earthworm occurrence

The first year, 2007 results were affected by drought (Map 2); the northern third of Wisconsin, was in either a severe or moderate drought for all of late summer. Most earthworm species aestivate during droughts, especially burrowing taxa such as *Lumbricus terrestris* (Bohlen and Hendrix 1995).



Drought Severity

D0 - Abnormally Dry

D1 Drought - Moderate

D2 Drought - Severe

D3 Drought - Extreme

D4 Drought - Exceptional

Drought Severity Index Map August 14, 2007

National Weather Service
http://droughtmonitor.unl.edu/archive.html

Map 2: Drought Severity August 14, 2007

Despite the severe drought of 2007, for all years the frequency of a sample capturing worms remained at 82%; for 2009 and 2010, it was higher at 90% (Table 1).

Table 1: Earthworm Occurrence by Year

Year	Sites Sampled	Worms Present Sites	Percentage Frequency
2007	39	27	69%
2009	36	32	88%
2010	26	24	92%
Total	101	83	82%

Earthworm occurrence: Lumbricus rubellus

The results show they are widespread in both forests; 88 % of sites in 2009 and 92% in 2010 (Figure 1).

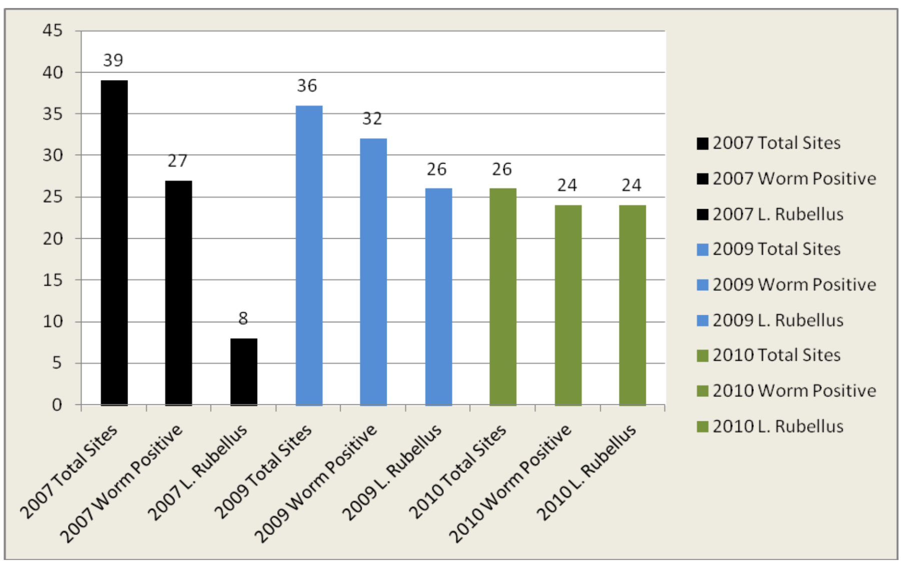


Figure 1: *Lumbricus rubellus* positive sites for all years

Earthworm occurrence: Lumbricus terrestris

Results for 2009 and 2010 show similar frequencies for *Lumbricus terrestris;* 55% in 2009 and 66% in 2010 (Figure 2).

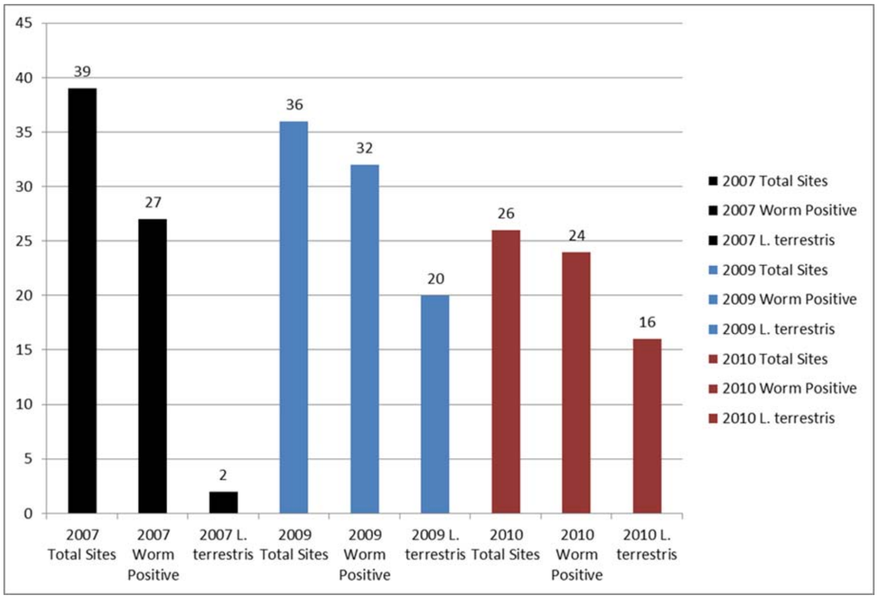


Figure 2: *Lumbricus terrestris* positive sites for all years

Earthworm stages of invasion

For 2009 -2010 data samples sites were evaluated by the four class invasion category system (Table 2). The frequency of occurrence, especially *Lumbricis rubellus*, shows much of the forest is already inhabited. Another important feature within the data is the majority of 2009 – 2010 sample sites are in late stage of invasion, as classes three and four account for 84.5% in 2009 and 82.2% of sample sites in 2010. The results indicate that both forests are already severely impacted.

Table 2: Sites by Frelich Earthworm Effect Category. Frequency and percentage of total sites. 2009 and 2010 data.

Year	Stage 1	Stage 2	Stage 3	Stage 4
2009 Occurrences	1	6	21	8
2010 Occurrences	1	3	12	10
2009 Percentage	2.7	16.6	58.3	22
2010 Percentage	3.8	11.5	46.1	38.4
Average Percentage (2009 and 2010)	3.22	14.5	53.2	29

Vegetation Quantitative Summary

An initial quantitative estimate was completed to use for later comparisons (Table 3). This was extracted using the mean percentage of cover classes to estimate percentage total cover. *Carex pensylvanica* was the most dominant understory species. While several species occur almost as frequently as *Carex pensylvanica*, the species cover is nearly a factor of ten over the next most common species (*Acer saccharum*). Other important species or species groups included other *Carex* species, Poaceae (grasses), *Fraxinus* seedlings, and *Aralia nudicaulis*.

Table 3: Vegetation Less than One Meter: Frequency, Estimated Mean Cover in square meters and Percentage Cover. 2007, 2009 —2010 Data. 101 Sites.

Taxa	Occurrences	Frequency	CoverM2	Cover Percentage
Carex pensylvanica	95	0.94	154.203	12.14
Acer saccharum	80	0.792	16.714	1.31
Maianthemum canadense	80	0.792	7.34	0.58
Trientalis borealis	65	0.643	0.657	0.051
Carex Unid	46	0.455	16.597	1.307
Polygonatum pubescens	41	0.405	1.117	0.092
Trillium ssp.	32	0.316	1.632	0.128
Dryopteris intermedia	31	0.306	8.874	0.7
Fern Unid	31	0.306	7.302	0.57
Aralia nudicaulis	30	0.297	11.698	0.921
Populus tremuloides	30	0.297	6.048	0.47
Dryopteris spp.	29	0.287	7.381	0.58
Fraxinus spp	28	0.277	15.46	1.21
Poaceae Unid	28	0.277	12.87	1.006
Eurybia macrophylla	18	0.178	7.067	0.55

Anderson-Darling test results indicate a highly non-normal distribution with a *P-value* of >0.0005 (Appendix H). Therefore, non-parametric methods are indicated.

NMDS Analyses of plant Community Composition

Nonmetric multi-dimensional scaling

To test the relationships between plant community composition, plant species or taxa were tested for responses to environmental variables including earthworms, deer browsing and earthworm invasion categories. A stable ordination in two dimensions was found with a stress of 18.20; the stress is relatively high, but highly dominant taxa, in this case *Carex pensylvanica*, may “skew” the distance relationships resulting in high stress (see Figure 3: Dominance Curve) (McCune and Grace 2002). The two axes accounted for 84% of the variation (Table 4). In this ordination, *Acer saccharum* responds negatively strongest to axis 1, while *Carex pensylvanica* is nearly equivalent positively across both axes. Table 5 is plant taxa correlations to the ordination axes, *Acer saccharum* and several herbs (*Maianthemum*, *Trillium*, *Dryopteris*, *Trientalis*), show somewhat similar responses, pointing to the possible existence of alternate *Carex pensylvanica* and *Acer saccharum* dominated communities.

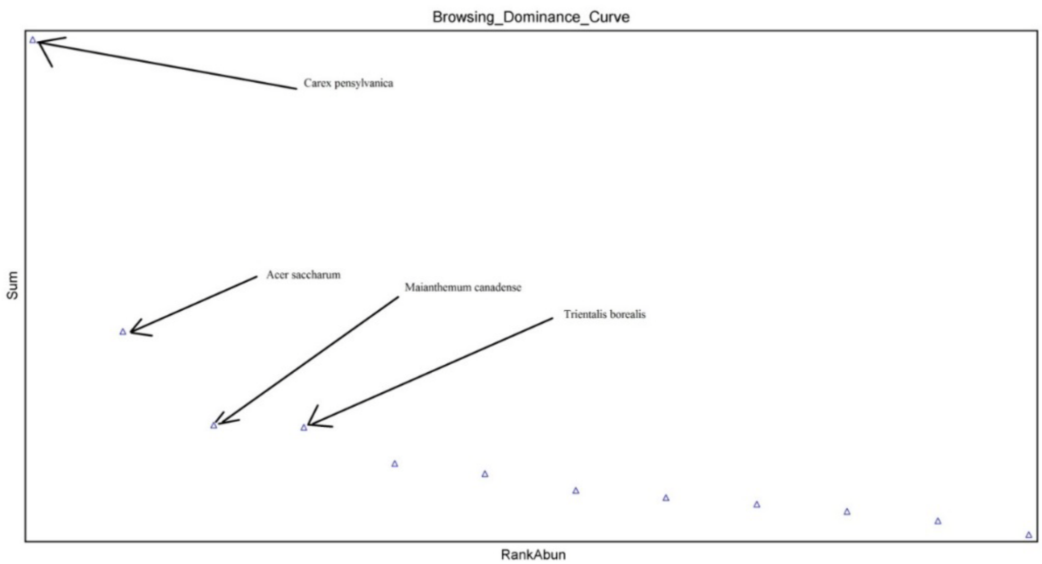


Figure 3: Dominance Curve: 2009 – 2010 Data: Raw Data scores. Taxa Minimum Fifteen Percent Occurrence. “X” axis is rank of abundance, with higher to lower values from left to right. Higher occurrence on the left in order of descending frequency to the right. Carex pensylvanica far upper left.

Table 4: Proportion of Variance explained by the first two NMDs axes (2009 – 2010 data fifteen percent site occurrence minimum).

Axis	Increment	Cumulative
1	0.378	0.378
2	0.462	0.84

Table 5: NMDS 2009 – 2010 Taxa Correlation by Axis: Kendall's Rank Correlation Coefficient. The correlation between the rank ordering of the data and the distance matrix. *Carex pensylvanica* and *Acer saccharum* respond in opposition, indicating divergence. Large effect size is 0.400.

Correlation Main Matrix
Kendall Tau Correlations with Ordination Axes N= 62

	Axis 1 Tau	Axis 2 Tau
Acer saccharum	**-0.704**	-0.316
Carex pensylvanica	**0.434**	**0.566**
Unidentified Carex	-0.198	-0.212
Dryopteris intermedia	0.023	0.115
Dryopteris species	-0.052	-0.311
Fraxinus species	0.038	-0.157
Maianthemum canadens	-0.267	**-0.455**
Ostyra virginiana	-0.079	-0.064
Unidentified poaceae	0.075	-0.011
Polygonatum pubescens	-0.276	0.113
Trientalis borealis	-0.084	-0.268
Trillium species	-0.128	-0.214

Carex pensylvanica's response dominates the ordination as the only species in one ordination region. Though *Lumbricus rubellus* sites are throughout the ordination with clustering on one side of the ordination and along the axis *for Carex pensylvanica* (Figure 4). *Lumbricus terrestris* sites are more dispersed and are less common (Figure 5). *Dendrobaena octaedra* sites are also dispersed, not dominating one region (Figure 6); the same is true for immature *Lumbricus* species (Figure 7). Non-pigmented species are largely in the same region dominated by *Lumbricus rubellus* and *Carex pensylvanica* (Figure 8). The study sites are populated largely by Frelich Classes 3 and 4, indicating the forest is heavily earthworm impacted (Figure 9). They are also heavily browsed as most sites are browsing class 3, or heavily browsed and dispersed throughout the ordination

(Figure 10). *Carex pensylvanica* dominated stands form a cluster separate from other taxa; the “Heavy” browse classification also appears to dominate within this same region.

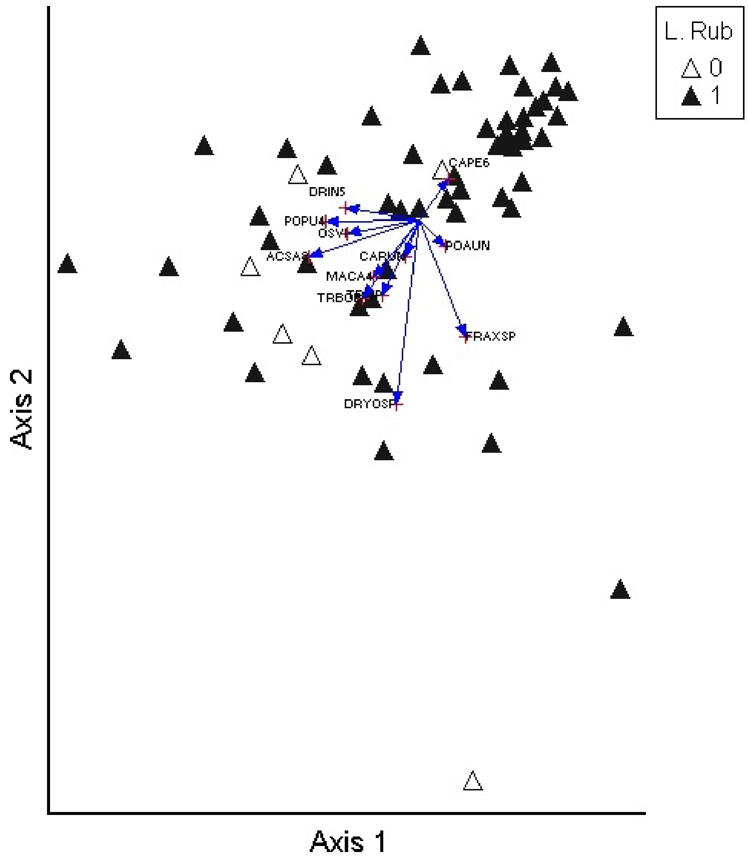


Figure 4: NMDS Fifteen Percent Taxa Removed *Lumbricus rubellus* positive. “1” or filled triangles indicate Lumbricus rubellus present.

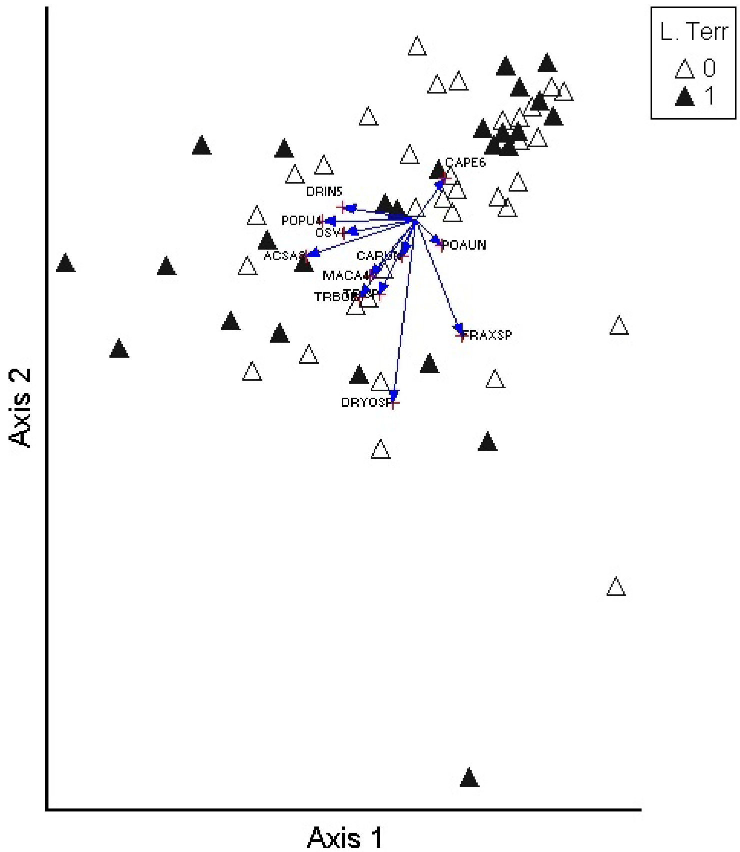


Figure 5: NMDS Fifteen Percent Occurrence Minimum *Lumbricus terrestris* positive sites. “1” or filled triangles indicate earthworm species present.

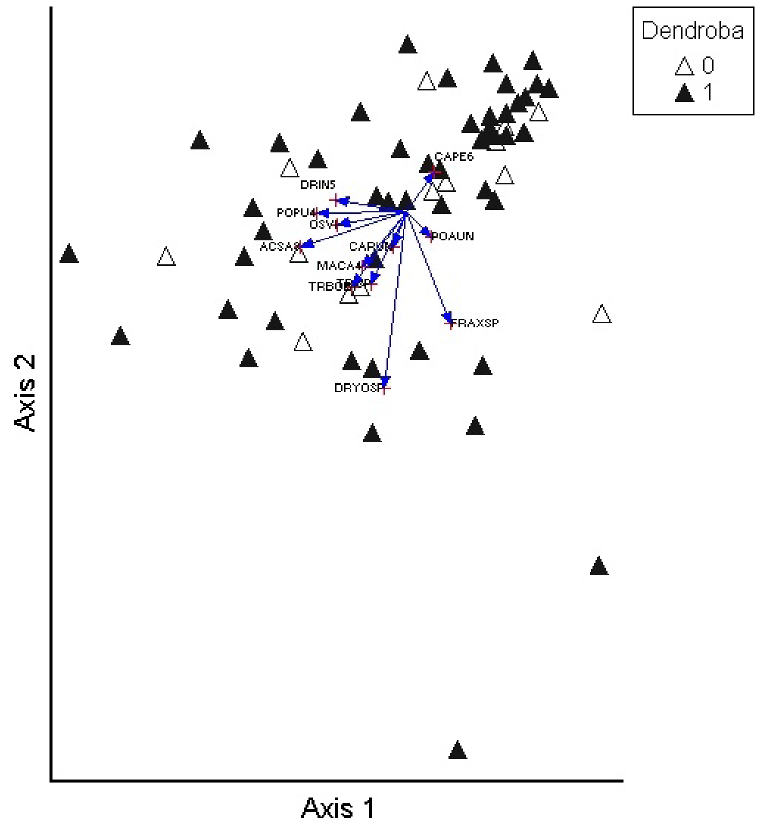


Figure 6: NMDS Fifteen Percent Minimum Occurrence *Dendrobaena octaedra* positive sites. “1” or filled triangles indicate earthworm species present.

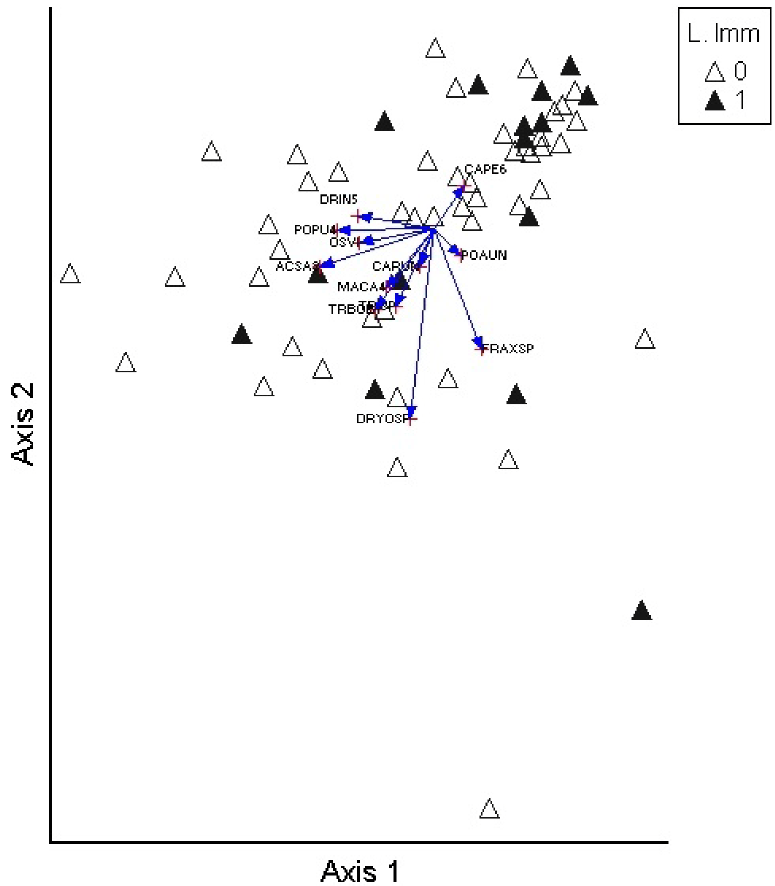


Figure 7: NMDS Fifteen Percent Minimum Occurrence Immature *Lumbricus* spp. Unidentified species specimens. “1” or filled triangles indicate earthworm species present.

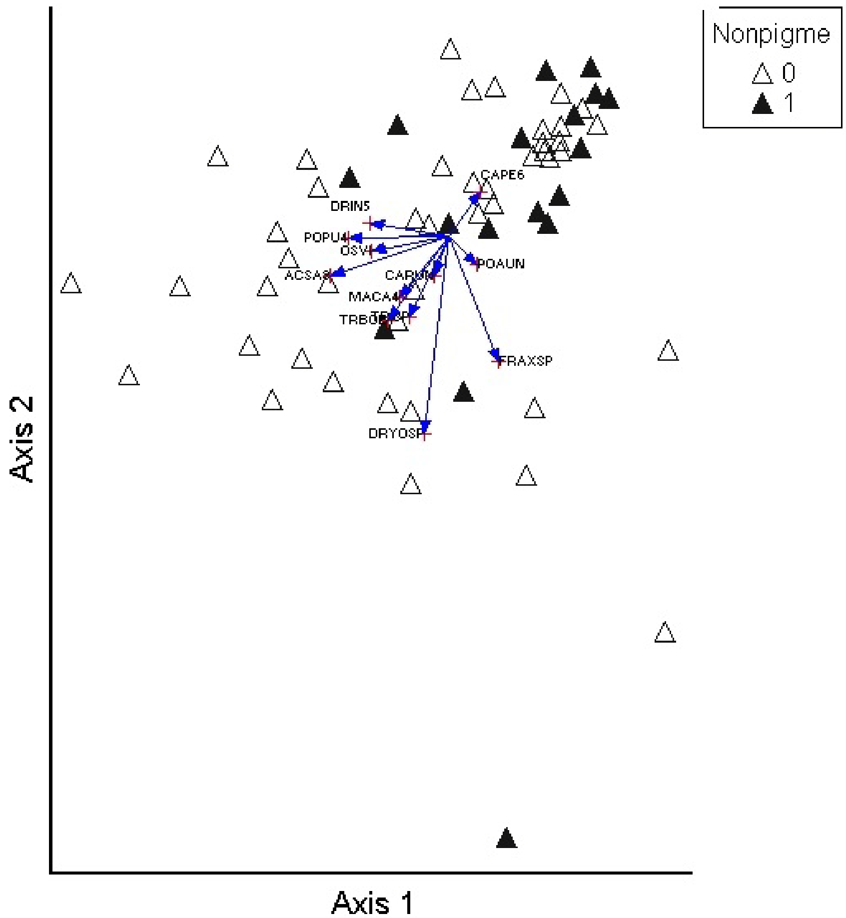


Figure 8: NMDS Fifteen Percent Minimum Occurrence. “1” or filled triangles indicate earthworm species present.

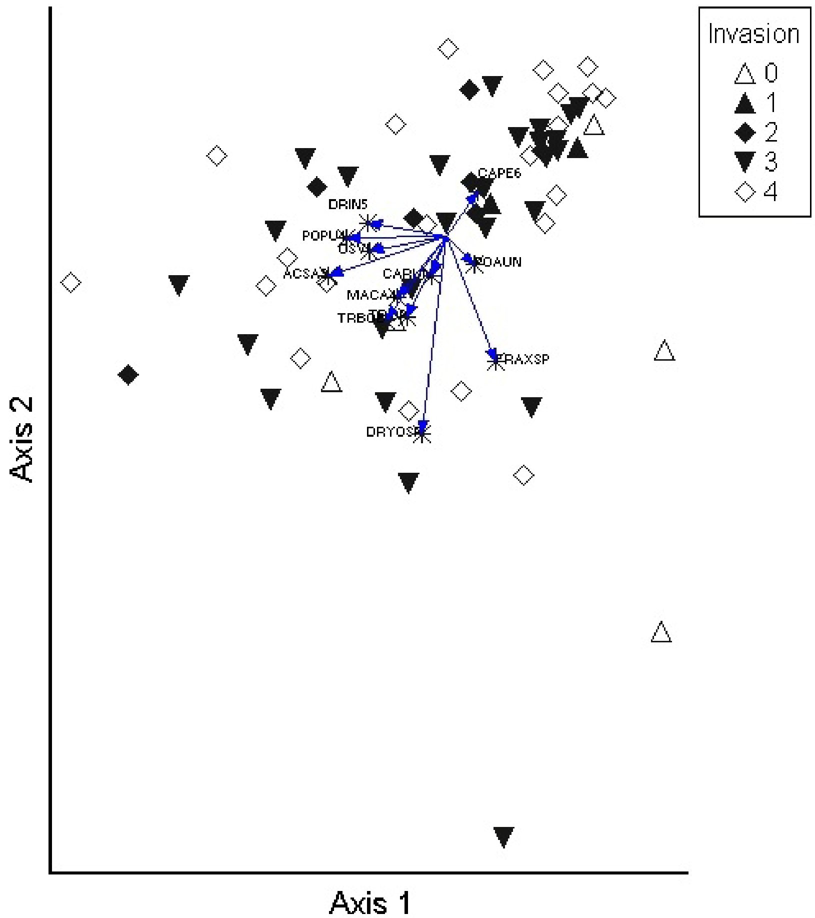


Figure 9: NMDS Fifteen Percent Minimum Occurrence Frelich Invasion Class. Sites overlaid by Frelich four category earthworm invasion effect scale. “0” sites are sites without a classification.

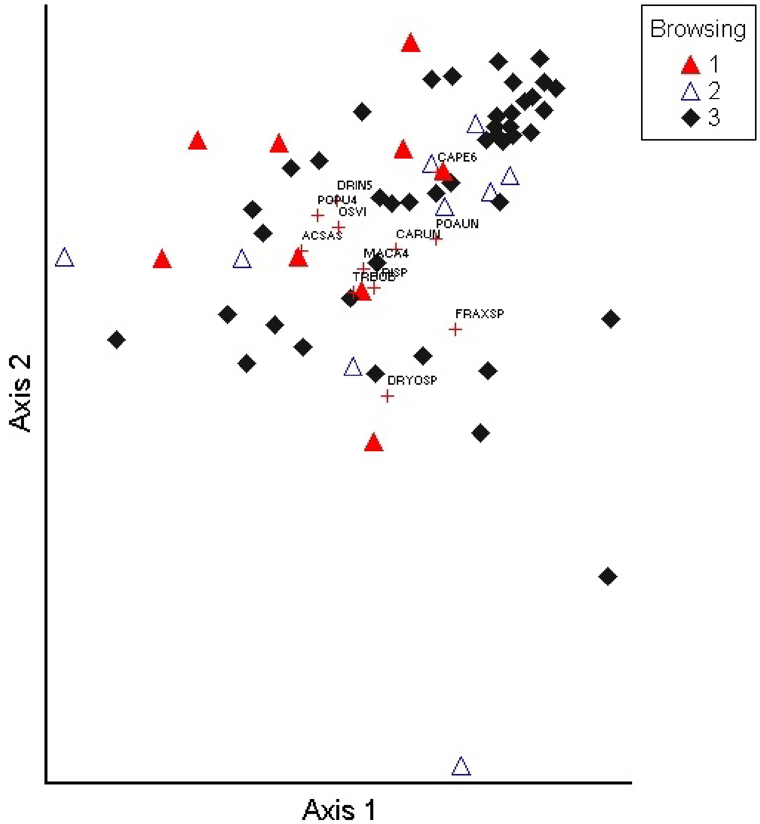


Figure 10: 2009-2010 Data Browsing NMDS. Classes are “1”=light; “2”= medium; “3” = heavy.

Plant taxa responses to environmental variables: Lumbricus terrestris

Figures 11 through 16 are overlay analyses of individual plant species or taxonomic group responses to *Lumbricus terrestris* presence by cover. Larger triangles mean greater cover for that individual species in a stand. All of the species shown had mixed results in that plots in which they occurred at relatively high abundance included plots with and without *Lumbricus terrestris* (i.e. the larger triangles in the figures are both black and white). *Acer saccharum* (Figure 11) and *Carex pensylvanica* (Figure 12) both appear with plots of high abundance equally split between *L. terrestris* present and absent, while unidentified *Carex* (Figure 13) and *Maianthemum* (Figure 14) have more plots without *L terrestris* present, and unidentified Poaceae (Figure 15) and *Trillium* (Figure 16), are difficult to interpret.

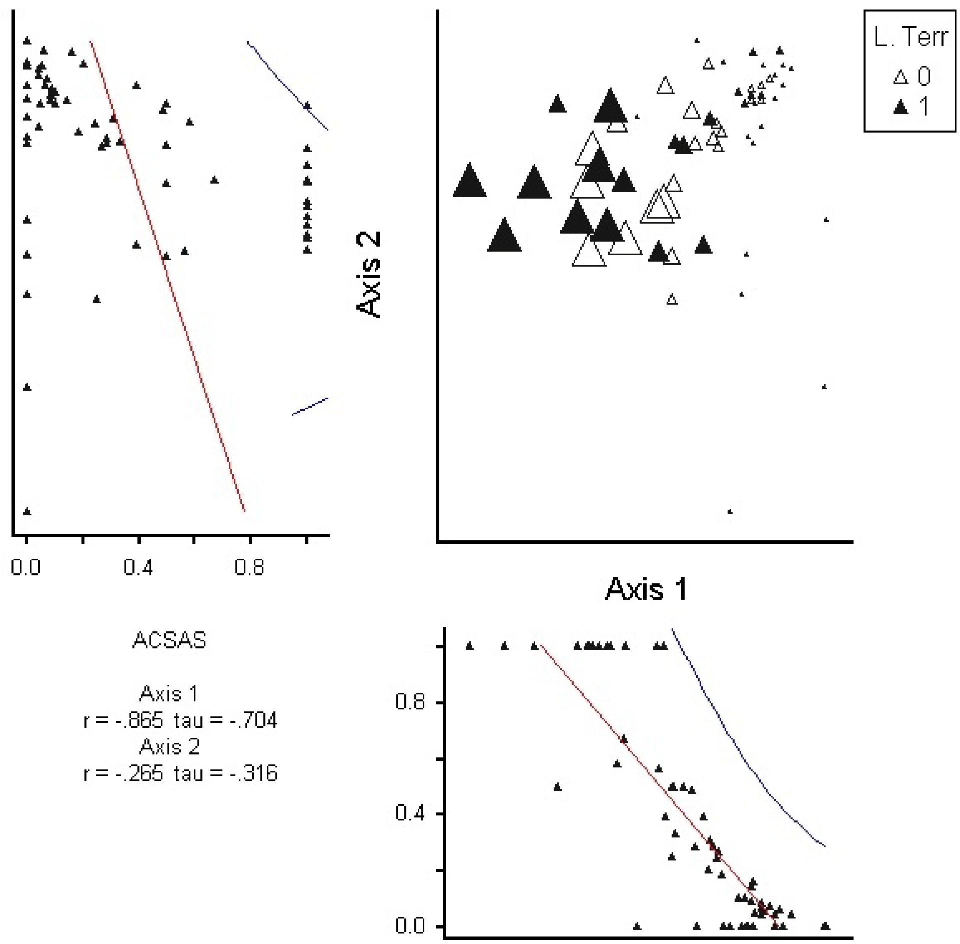


Figure 11: *Acer saccharum* response (indicated by size of triangles) to *Lumbricus terrestris*. “1” or filled triangles indicate earthworm species present.

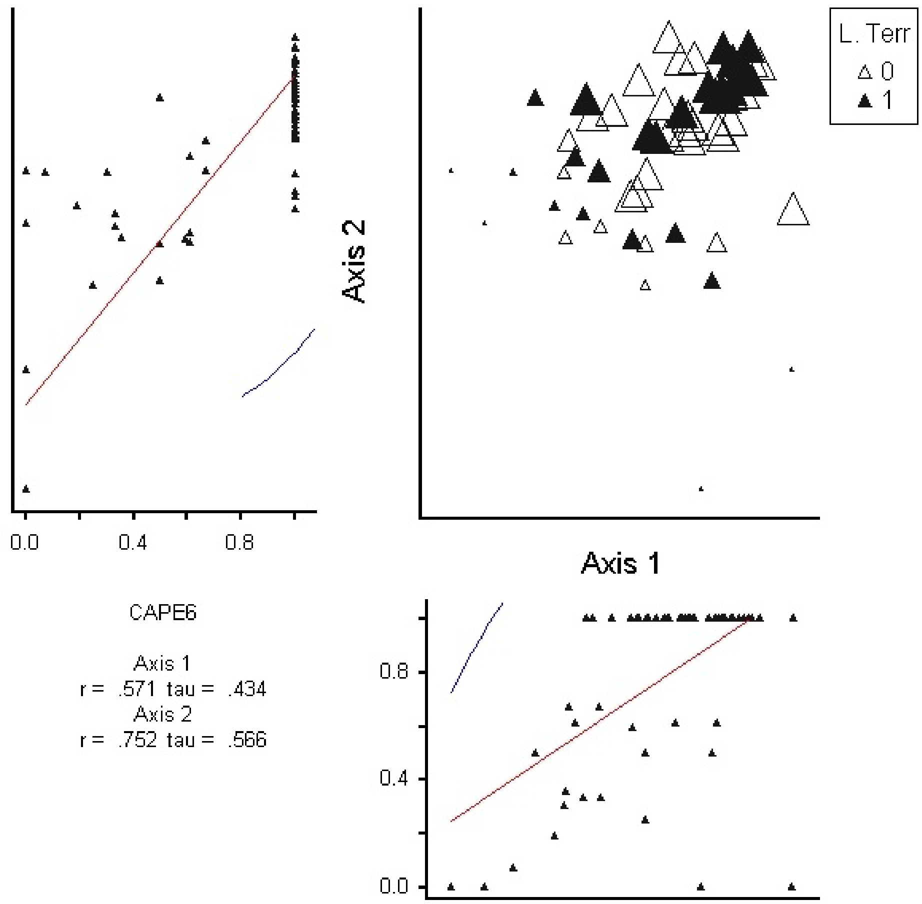


Figure 12: *Carex pensylvanica* response (indicated by size of triangles) to *Lumbricus terrestris*. "0" values are negative for *Lumbricus terrestris*. "1" or filled triangles indicate earthworm species present.

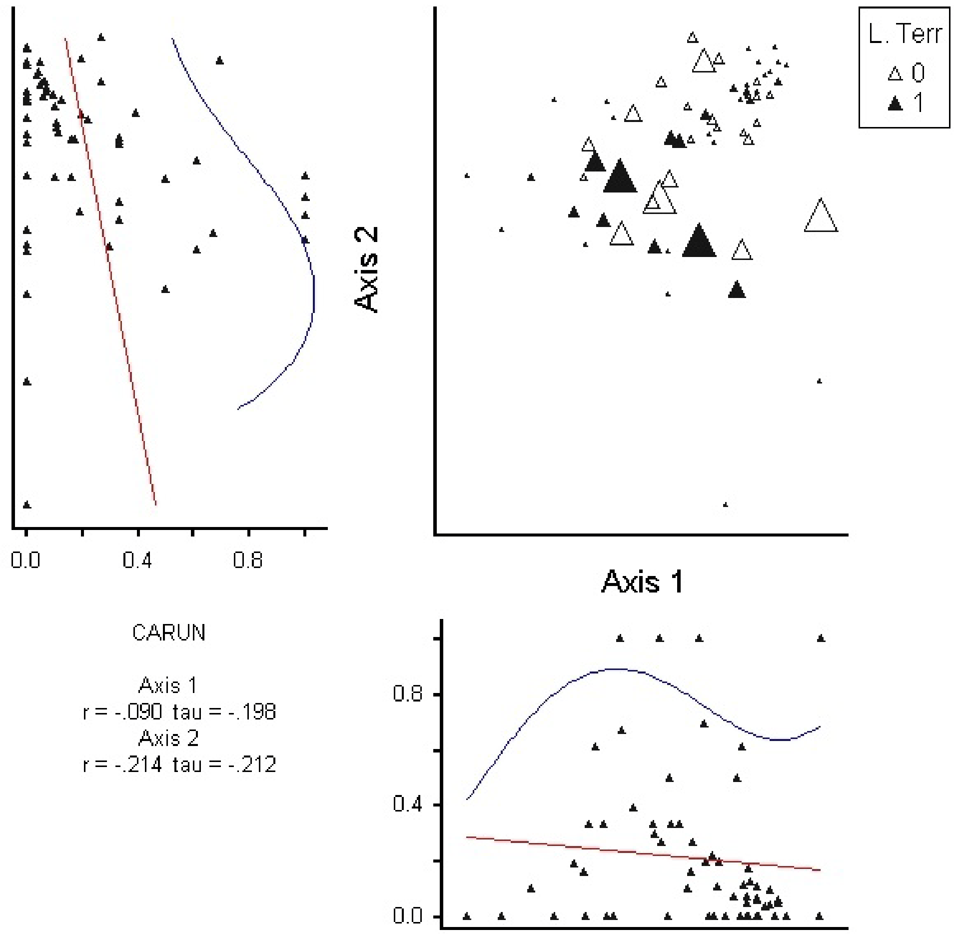


Figure 13: Unidentified *Carex* species response (indicated by size of triangles) to *Lumbricus terrestris.* Individual *Carex* species response may differ. “1” or filled triangle indicates *Lumbricus terrestris* present.

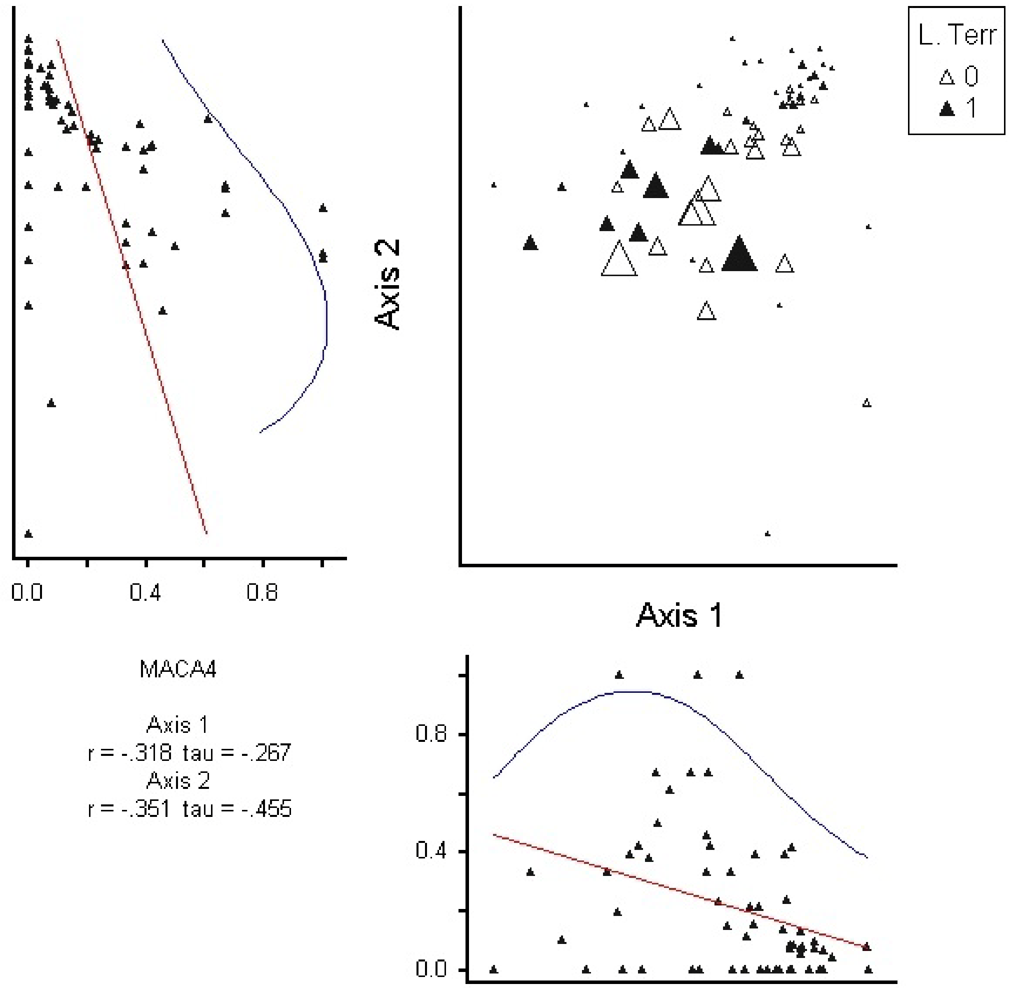


Figure 14: *Maianthemum canadense* response (indicated by size of triangles) to *Lumbricus terrestris*. “1” or filled triangle indicates *Lumbricus terrestris* present.

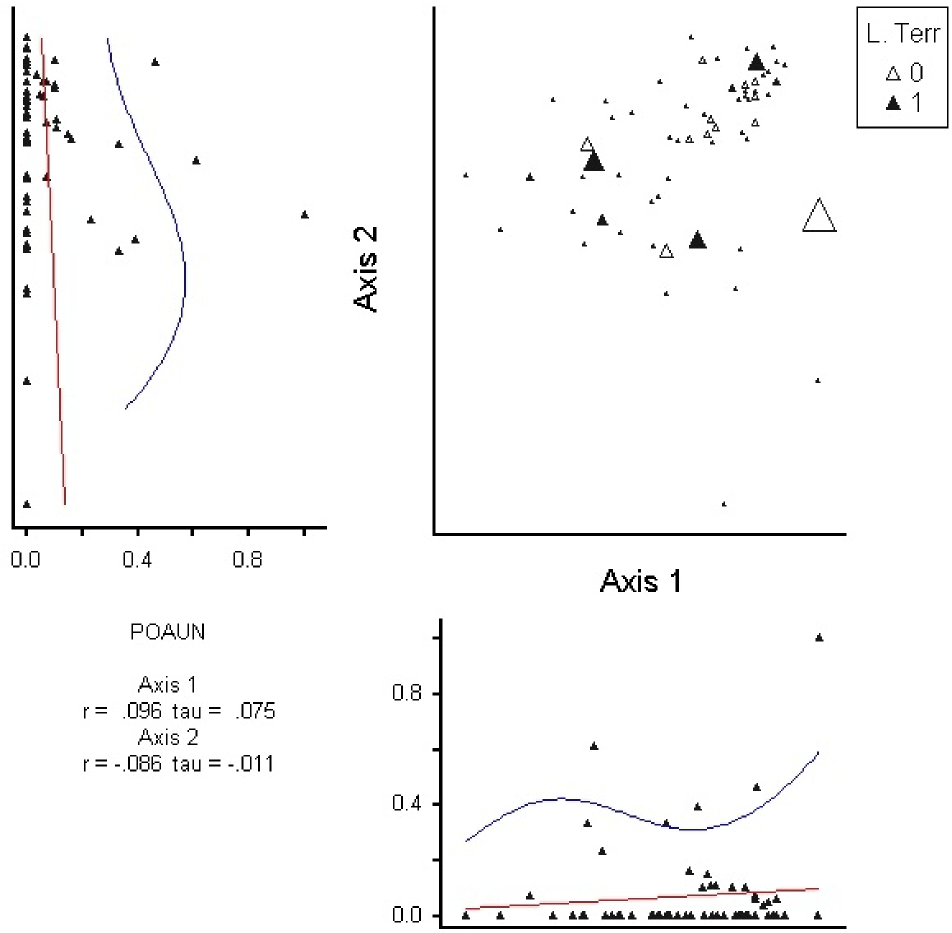


Figure 15: Unidentified Poaceae response (indicated by size of triangles) to *Lumbricus terrestris*. Individual species response may differ. “1” or filled triangle indicates *Lumbricus terrestris* present.

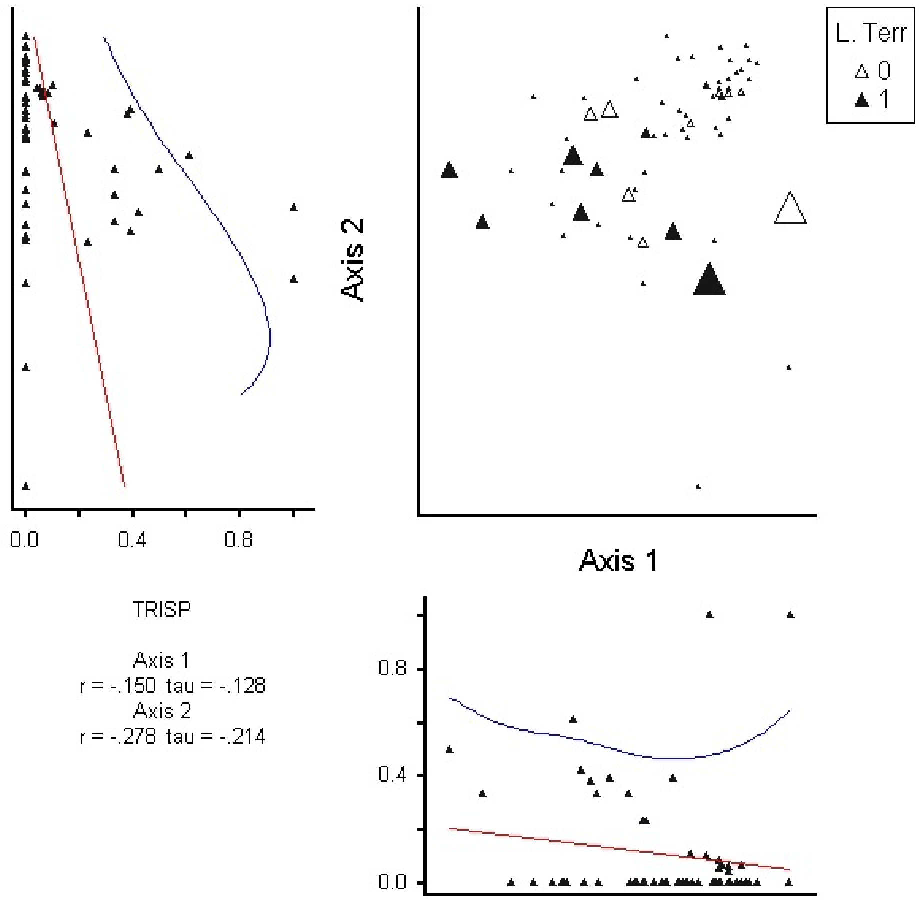


Figure 16: *Trillium spp.* response (indicated by size of triangles) to *Lumbricus terrestris*. Individual species responses may differ. “1” or filled triangles indicates *Lumbricus terrestris* present.

Plant taxa responses to environmental variables: Lumbricus rubellus

Figures 17 through 22 are overlay analyses of individual plant species or taxonomic group responses to *Lumbricus rubellus* presence. Because this earthworm species was present on most plots, it was difficult to detect patterns of plant species response. Larger triangles mean greater cover for that individual species in a stand *Acer saccharum* for example, had no clear response but it appears there is a tendency for greater cover where there are Lumbricus rubellus free sites (Figure 17). Sites with *Carex pensylvanica* had a preponderance of high abundance *Lumbricus rubellus* locations (Figure 18). The remaining taxa, Unidentified *Carex* (Figure 19), *Maianthemum* (Figure 20), Unidentified Poaceae (Figure 21) and *Trillium* species (Figure 22) had ambiguous responses.

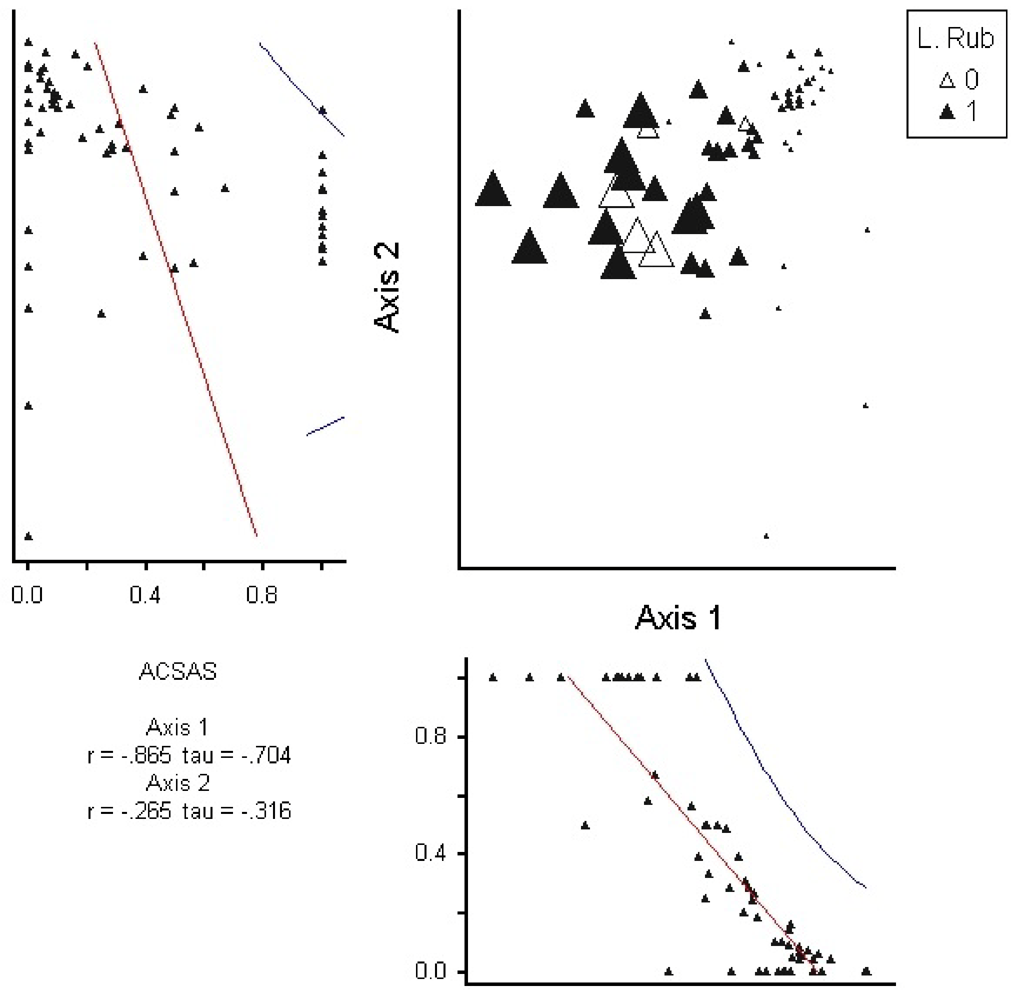


Figure 17: *Acer saccharum* response (indicated by size of triangles) to *Lumbricus rubellus*. “0” or empty triangles = *Lumbricus rubellus* not present; “1” or filled triangles = *Lumbricus rubellus* present.

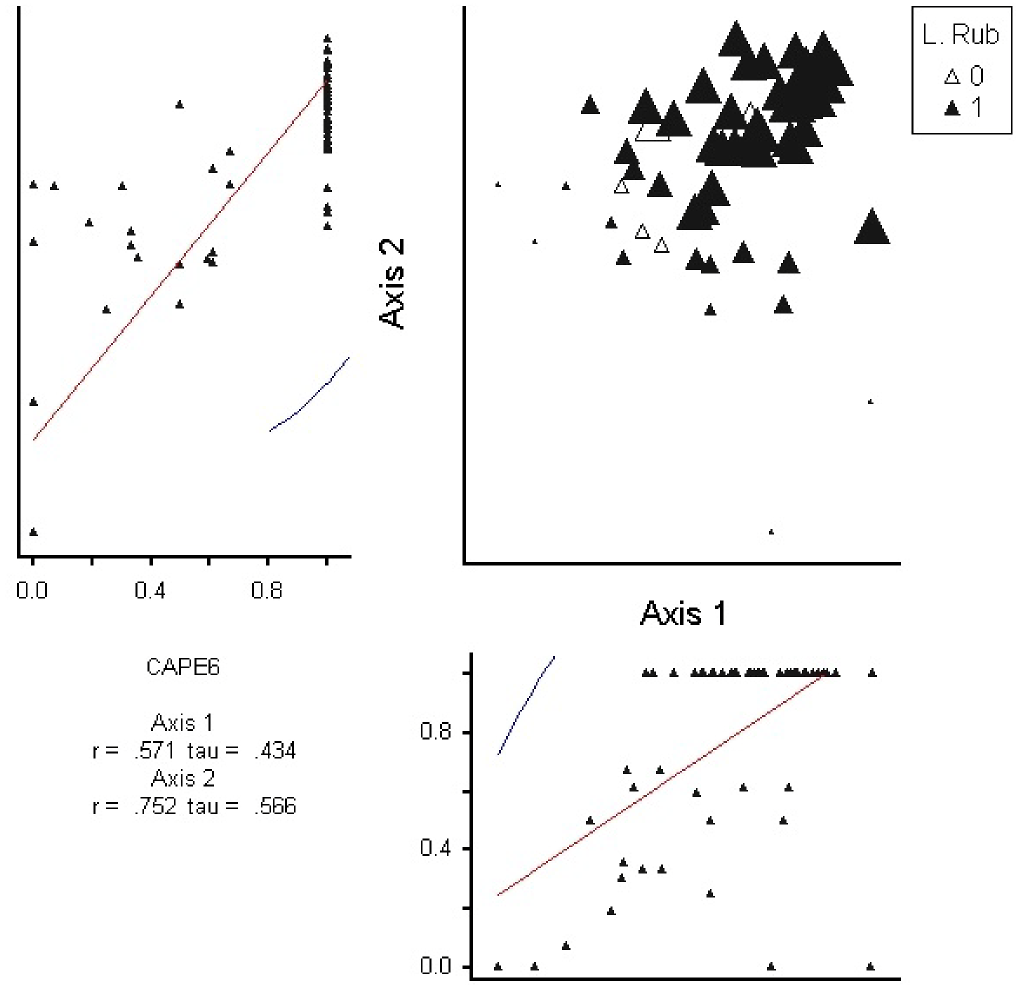


Figure 18: *Carex pensylvanica* response (indicated by size of triangles) to *Lumbricus rubellus*. 0" values as empty triangles = *Lumbricus rubellus* not present; "1" or filled triangles = *Lumbricus rubellus* present.

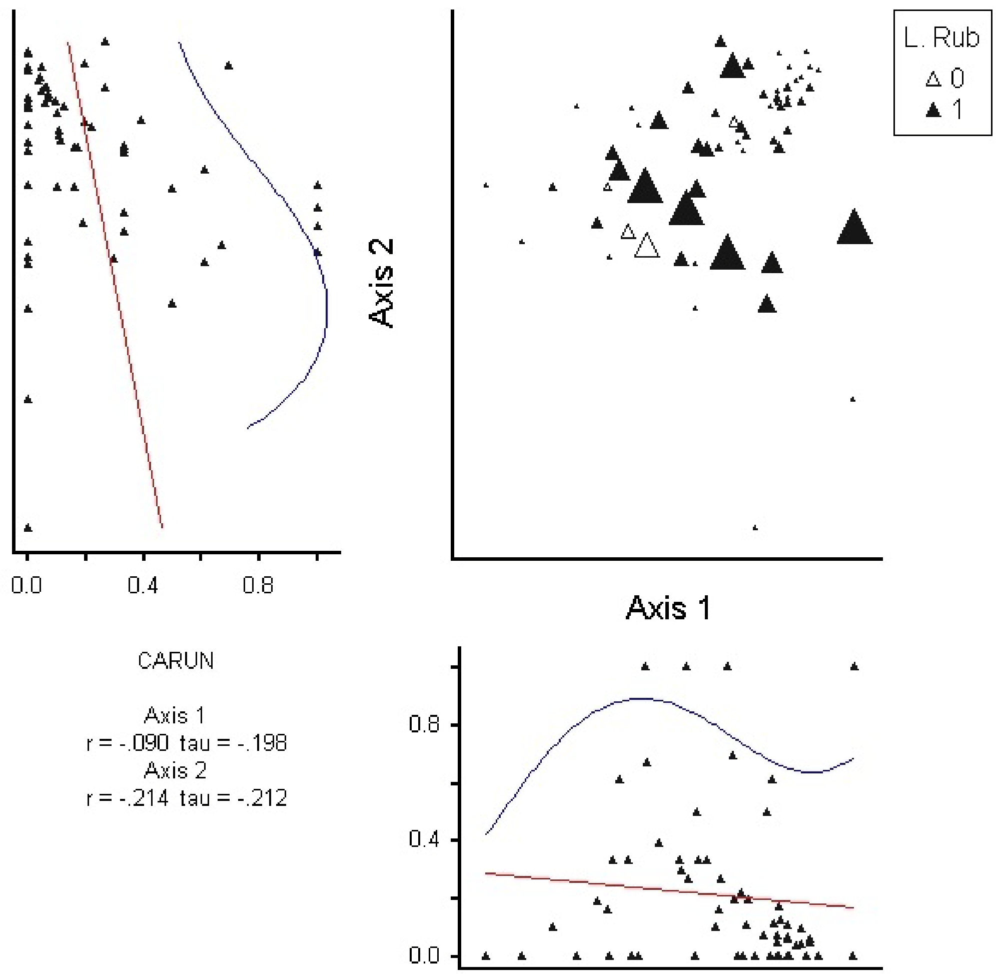


Figure 19: Unidentified *Carex* species response (indicated by size of triangles) to *Lumbricus rubellus*. “0” values as empty triangles = *Lumbricus rubellus* not present; “1” or filled triangles = *Lumbricus rubellus* present. Individual species responses may differ.

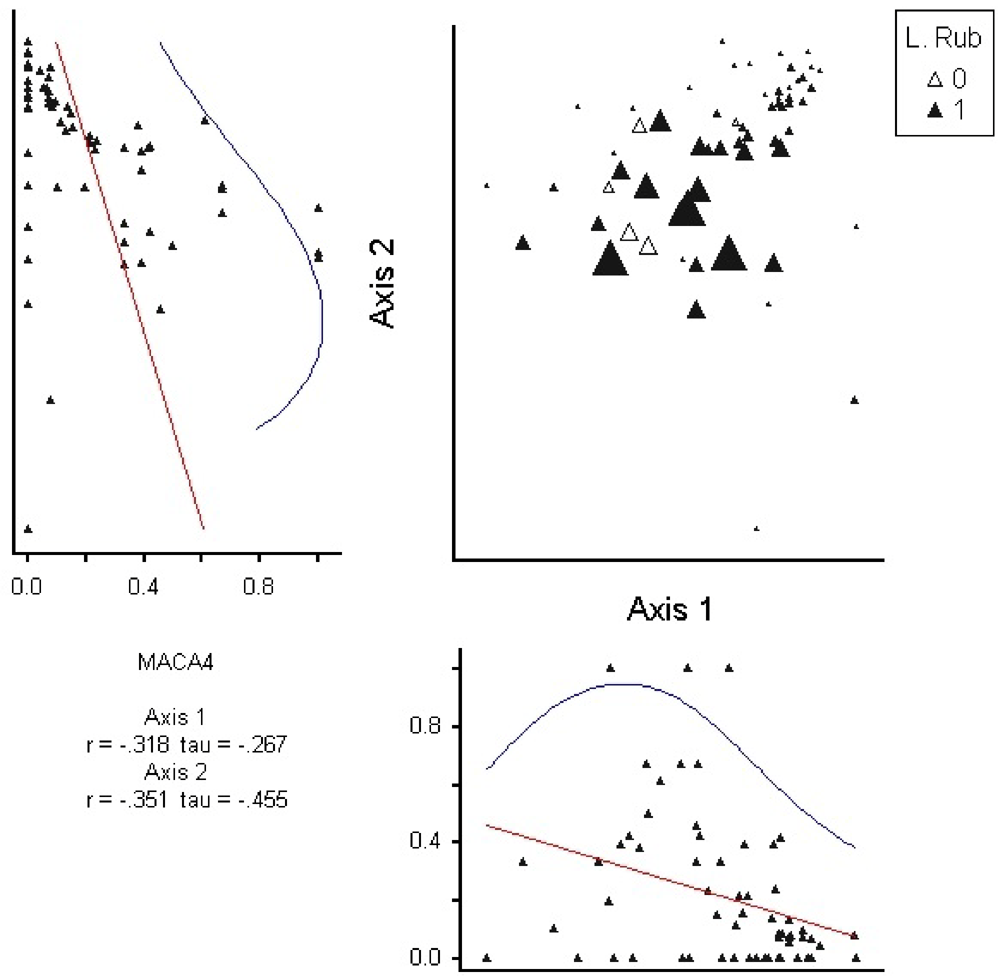


Figure 20: *Maianthemum canadense* response (indicated by size of triangles) to *Lumbricus rubellus*.”0” values as empty triangles = *Lumbricus rubellus* not present; “1” or filled triangles = *Lumbricus rubellus* present.

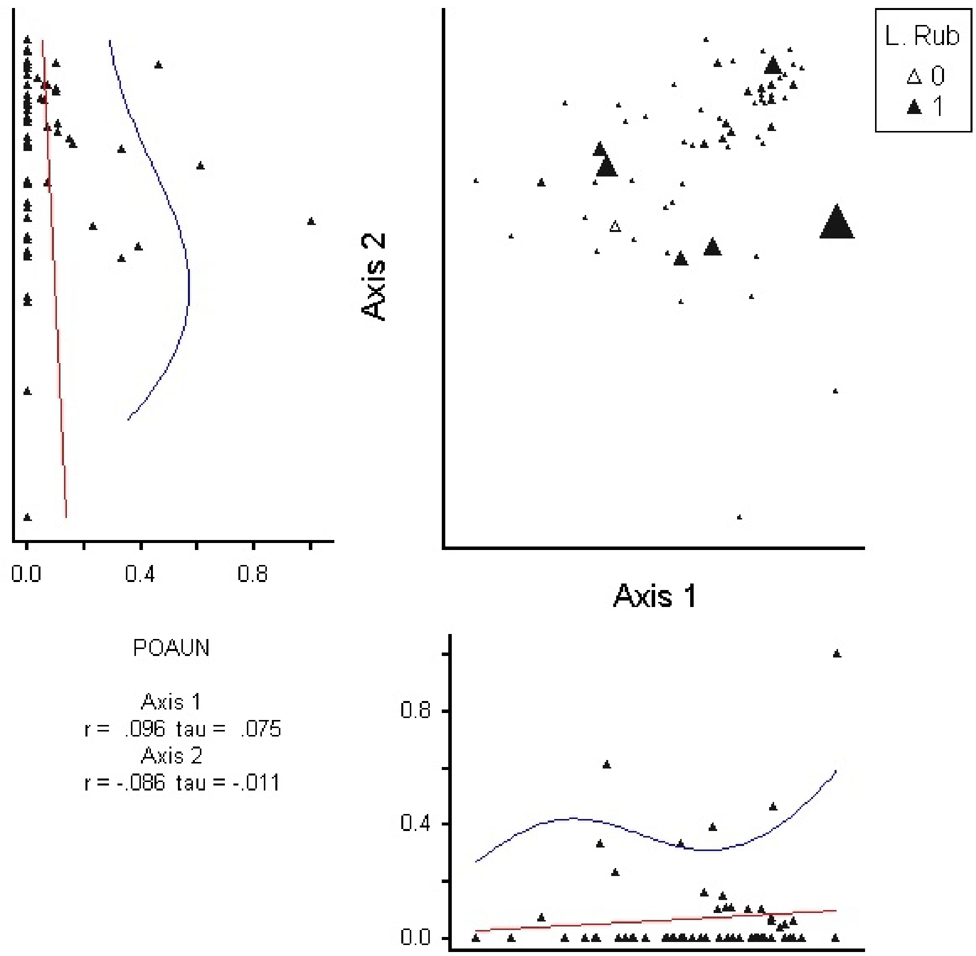


Figure 21: Unidentified Poaceae response (indicated by size of triangles) to *Lumbricus rubellus*. “0” values as empty triangles = *Lumbricus rubellus* not present; “1” or filled triangles= *Lumbricus rubellus* present. Individual species responses may differ.

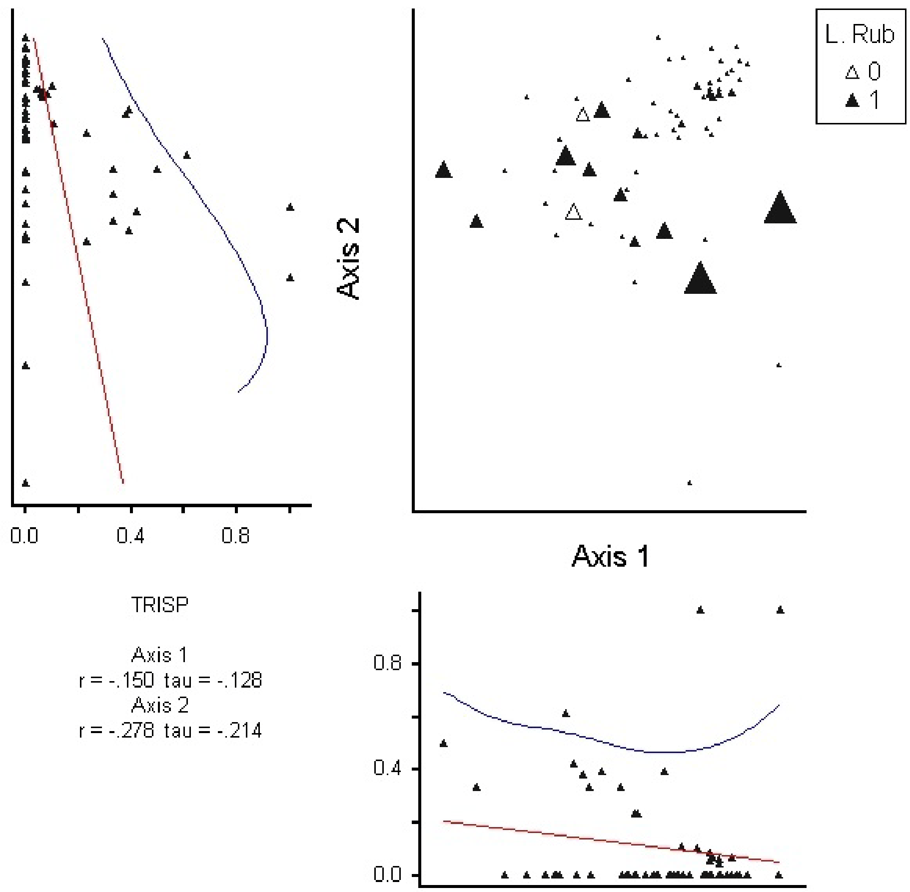


Figure 22: *Trillium* Species response (indicated by size of triangles) to *Lumbricus rubellus*. "0" or empty triangles = *Lumbricus rubellus* not present; "1" or filled triangles = *Lumbricus rubellus* present.. Individual species responses may differ.

In summary of the ordination analyses, despite the difficulties of interpreting the effects of individual earthworm species and plant species interactions, the results showed a strong relationship between *Carex pensylvanica*, presence of *Lumbricus rubellus*, stage of earthworm invasion (i.e. more earthworm species present means more *Carex pensylvanica*) and magnitude of deer browsing (Also see additional deer browsing results in the following section).

White–tailed deer browsing

Browsing Nonmetric Multi-dimensional Scaling

Plant taxa were compared against browsing as a three category variable, light, medium and heavy. The majority of sites were heavily browsed and so testing for comparison is difficult. *Carex pensylvanica* responds strongest positively to browsing (Figure 23) while *Acer saccharum* shows a strong negative response (Figure 24); unidentified Poaceae (Figure 25), unidentified *Carex* (Figure 26) and *Trillium* species (Figure 27) all show weak responses.

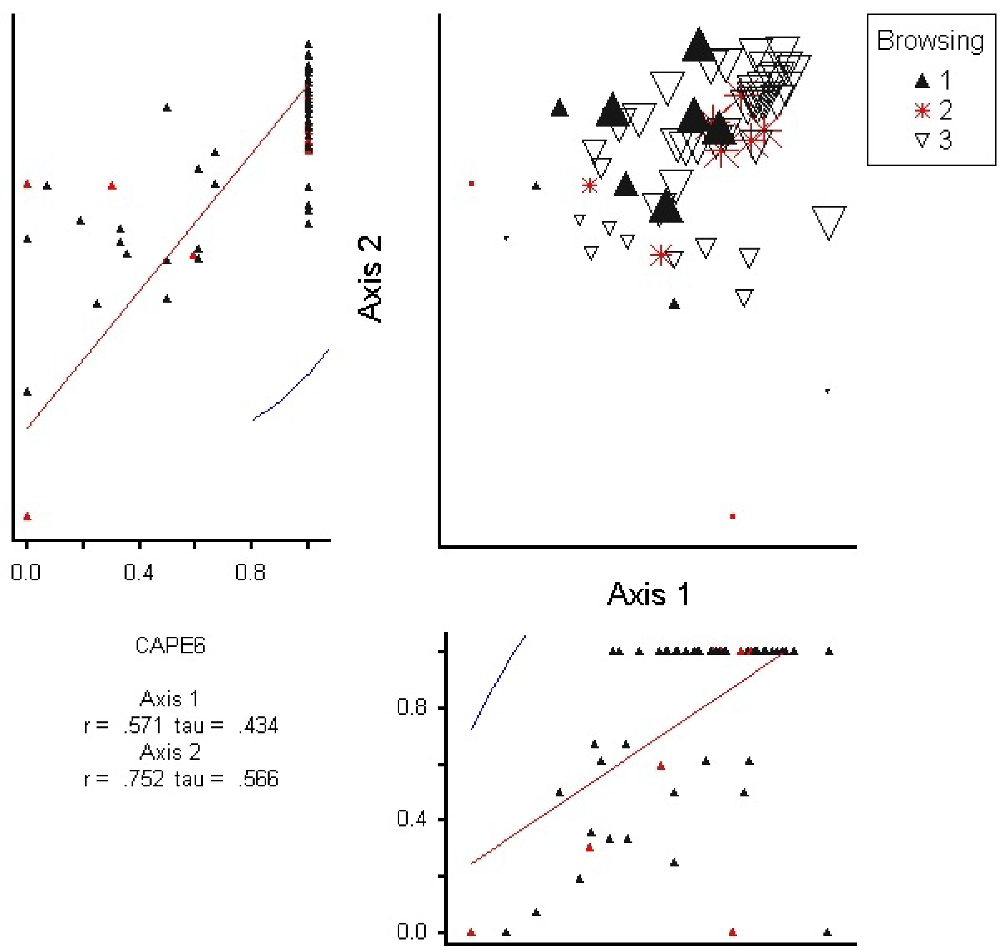


Figure 23: *Carex pensylvanica* response (indicated by symbol size) to Browsing. Classes are “1”=light; “2”= medium; “3” = heavy

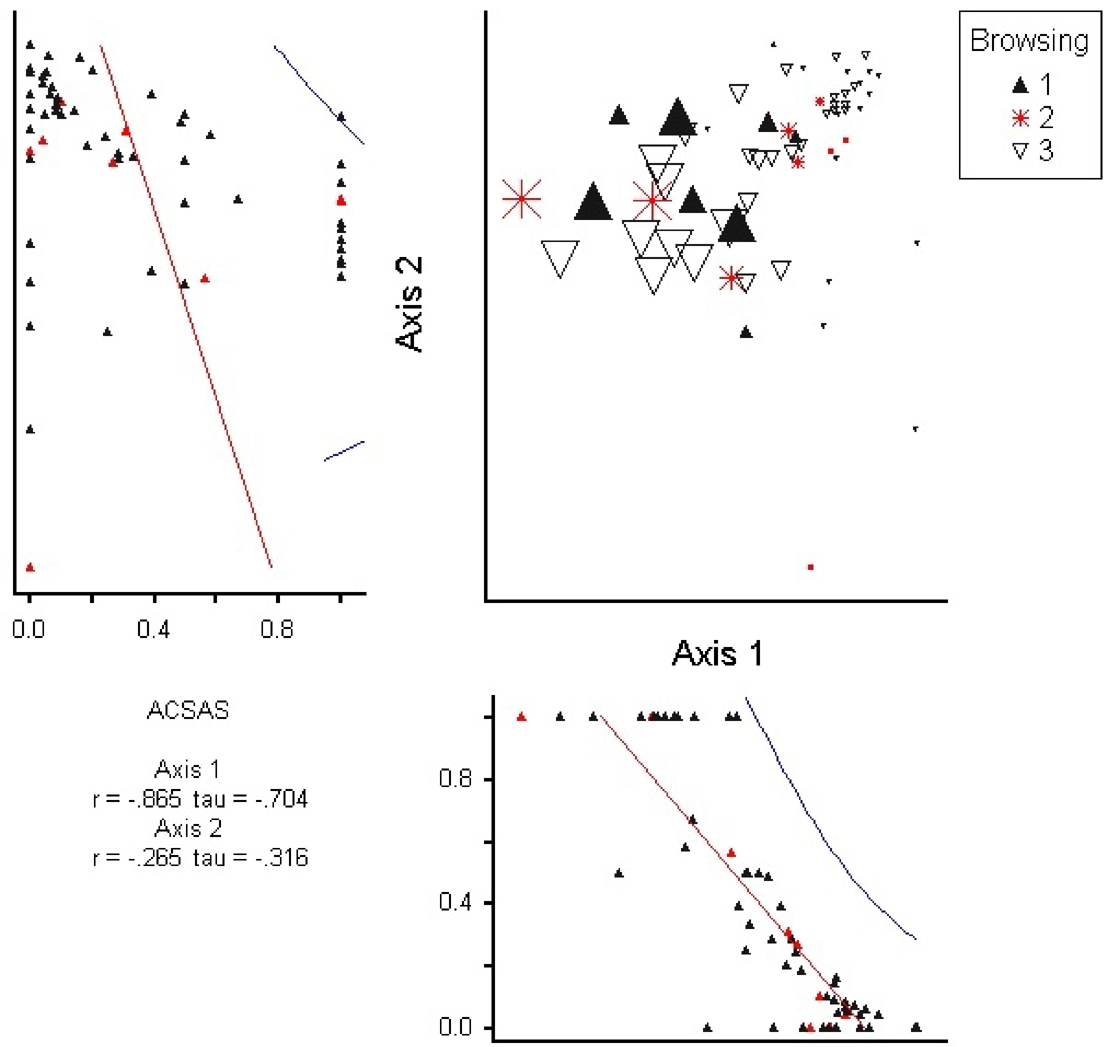


Figure 24: *Acer saccharum* response (indicated by symbol size) to browsing. Three classes: Classes are “1”=light; “2”= medium; “3” = heavy.

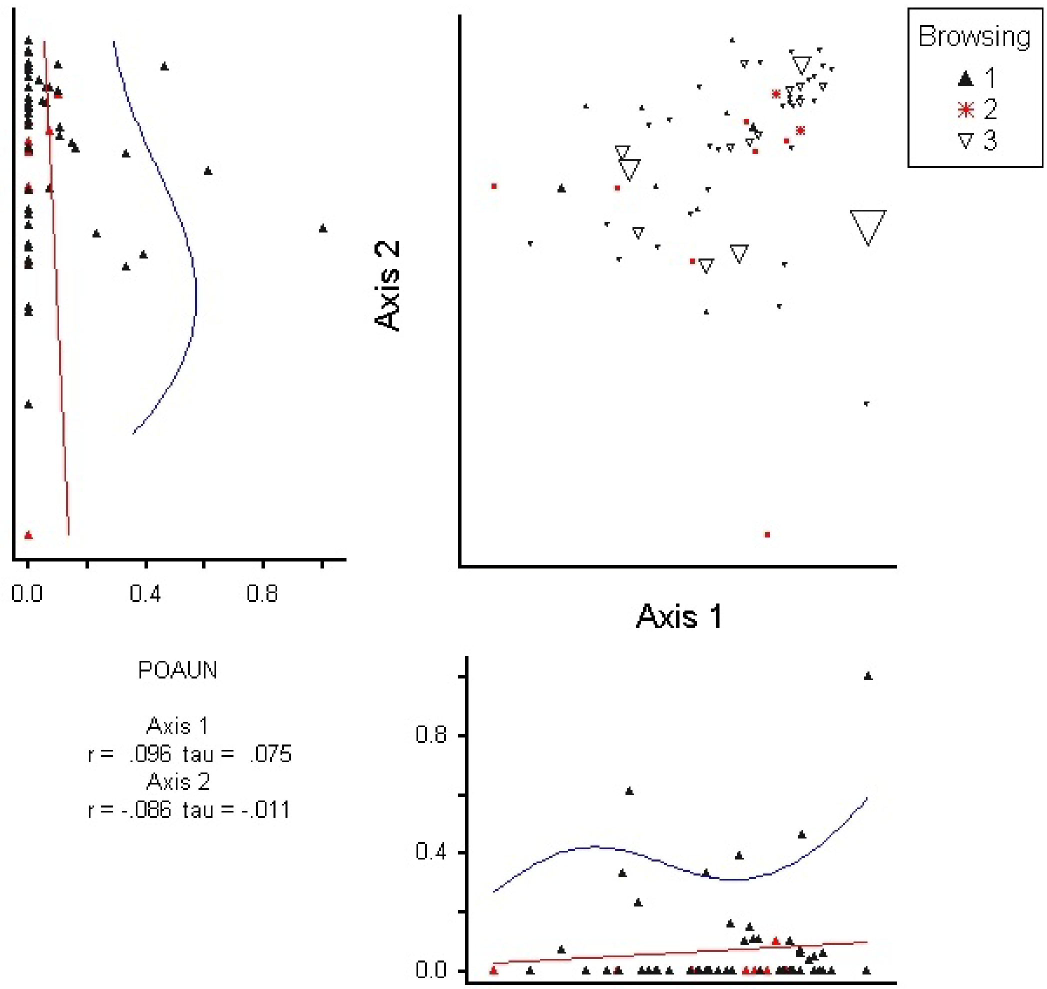


Figure 25: Unidentified Poaceae response (indicated by symbol size) to browsing. Classes are "1"=light; "2"= medium; "3" = heavy. Individual species responses may differ.

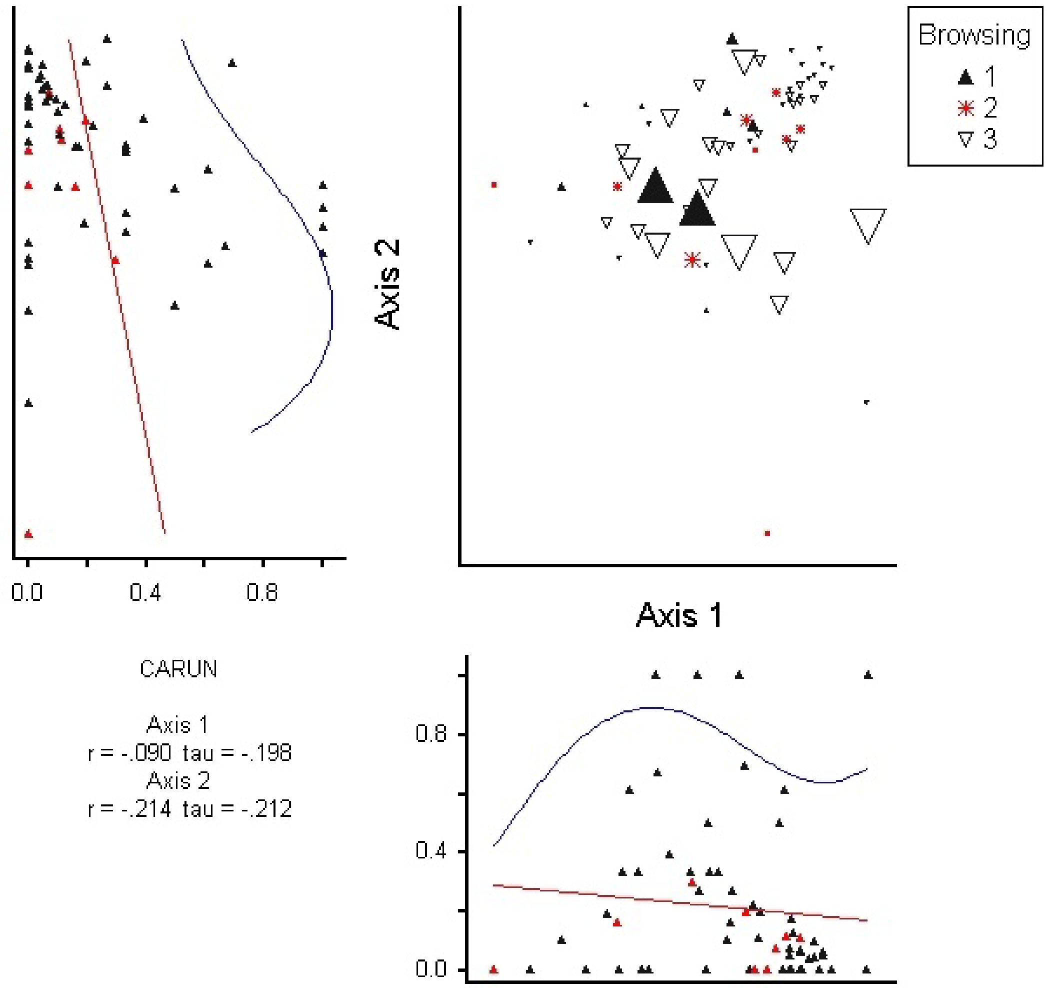


Figure 26: Unidentified *Carex sp.* response (indicated by symbol size) to browsing. Classes are “1”=light; “2”= medium; “3” = heavy. Individual species responses may differ.

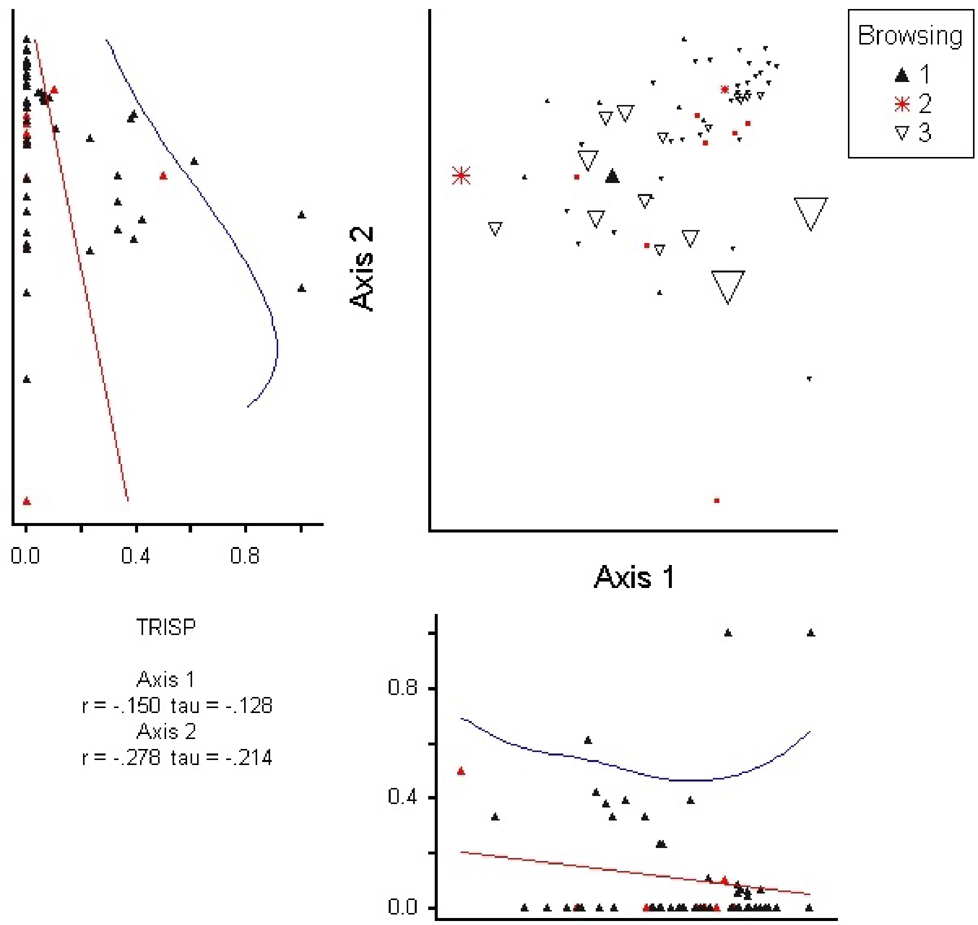


Figure 27: *Trillium* Species response (indicated by symbol size) to browsing. Classes are "1"=light; "2"= medium; "3" = heavy. Individual species responses may differ.

Indicator Species Analysis; Species Richness; Multiple Response Permutation Procedure

The full data set (2007, 2009 and 2010) was tested by the four category “Earthworm Effect” classification system. The 2007 data was drought affected and worm sampling results were reduced, leaving group classes limited. The 2009 – 2010 data had better earthworm sampling results and allowed for categorical comparison by earthworm species. Previous tests showed a relationship between *Carex pensylvanica* and *Lumbricus rubellus*, thus *Lumbricus rubellus* was chosen as the first categorical test. The results show *Carex pensylvanica* and *Lumbricus rubellus* as strongly linked with an Indicator value of 87 (Table 7). No other taxa showed higher indicator values, and although *Dryopteris* spp and *Aralia nudicaulis* had significant negative *P-values*, their indicator values were low (Table 7). Table 8 is Indicator Species Analysis in relation to Lumbricus terrestris. Only *Trillium* species had a significant *p value*, but its indicator value was small. Species richness curve estimation showed the *Acer saccharum* region sites had overall greater species richness; sites showed greater mean % cover, greater diversity and higher estimated asymptotic species richness (Table 9). The Multiple Response Permutation Procedure (MRPP) result shows two widely divergent communities as seen in the ordination diagrams (Table 10).

Table 6: Indicator Species: *Lumbricus rubellus* – Plant taxa relationship. The relationship of *Carex pensylvanica* with *Lumbricus rubellus* is strong with a significant value. Only the top ten frequently occurring taxa are shown to save space.

Indicator Values	Lumbricus rubellus				
Taxon	Average Groups	Maximum Member	Maximum Group	Indvalue	"P" Value
Carex pensylvanica	47	87	Positive	87	0.0312
Acer saccharum	41	55	Negative	26	0.4245
Carex undientified	34	37	Positive	37	0.9450
Maianthemum candanense	37	38	Positive	38	0.7978
Poaceae Unidentified	17	32	Positive	32	0.2883
Trientalis borealis	24	43	Positive	43	0.8256
Dryopteris spp	28	47	Negative	9	0.0338
Trillium spp	17	19	Negative	14	0.8256
Aralia nudicaulis	21	38	Negative	5	0.0488
Carex radiata	8	16	Negative	0	0.1036

Table 7: Indicator Species: *Lumbricus terrestris* – Plant taxa relationship

Indicator Values	Lumbricus Terrestris				
Taxa	Average Groups	Maximum Member	Maximum Group	Indvalue	"P" Value
Carex pensylvanica	46	47	Negative	47	0.9728
Acer saccharum	41	54	Positive	28	0.3147
Carex undientified	35	46	Negative	46	0.4179
Maianthemum candanense	35	44	Negative	44	0.2246
Poaceae Unidentified	15	22	Positive	7	0.8872
Trientalis borealis	27	27	Positive	26	0.9384
Dryopteris spp	17	21	Negative	21	0.6159
Trillium spp	20	33	Positive	7	0.0370
Aralia nudicaulis	12	17	Negative	17	0.3441
Carex radiata	3	7	Positive	0	0.4621

Table 8: Species Richness analyses of *Carex pensylvanica* and *Acer saccharum* communities. Ordination Region Site Diversity Estimates. *Acer saccharum* region group showed greater real and possible richness and diversity.

Ordination region	Carex pensylvanica	Acer saccharum
Mean (cover)	0.3616	0.7300
Stand Dev	0.2646	0.3719
Mean Taxa	12.1	13.8
H/Ln E	0.367	0.835
Shannon H	0.898	2.148
Simpson D	0.3683	0.8097
Mean Taxa	73	89
1st Order Jackknife	96.9	126.2
2nd Order Jackknife	103.1	147.8

Multiple Response Permutation Procedure

The results indicate the two community groups are distinct and highly separated. The "T" statistic measures separation between the groups, with greater negativity indicating higher separation. The "p" value indicates the probability that "T" occurred by chance and here is negligible. The value for the "A" statistic indicates within group agreement with values between "0" and "1", "0" meaning there is no within group agreement and "1" indicating perfect agreement (homogeneity); 0.36 is considered very high for ecological data (Table 9) (McCune and Grace 2002).

Table 9: Multiple Response Permutation Procedure

Carex pensylvanica region Avg Distance	0.18837072
Acer saccharum region Avg Distance	0.44987756
Chance corrected within group agreement	A= 0.36175172
P Value: Probability of Smaller or Equal Delta	0.00000
T =	-22.995489
Observed delta	0.31912414
Expected delta	0.50000000

Discussion

This study's results concur with previous findings for earthworm invasion extent and severity, both within the Chequamegon-Nicolet National Forest and the entire western Great Lakes. They indicate the forest floor plant community is transitioning from a more diverse "*Acer saccharum*" community state, to a simplified alternate Graminoid-Sedge dominated state with differing species at a broad scale (Wiegmann and Waller 2006*).* The 2007 drought likely altered results for earthworms as *Lumbricus terrestris* and other earthworm species aestivate during dry soil conditions (Hendrix 1995). The 2007 sites were randomly selected from the same database as the other years and it is fair to assume a similar occurrence frequency. If the overall frequency is similar (~ 50%) then large areas of the forest are already colonized by *Lumbricus terrestris*. If the same frequency rate held true for 2007 as the other years there would be another 20 or so sites with *Lumbricus terrestris* included in this study. That *Lumbricus terrestris* appears in 50% of samples for two non-drought years is significant as they appear *after* soil and vegetative changes have already occurred. The *Lumbricus rubellus* results indicate the species is long since well established and is common across the landscape of both forest units.

Restating the hypotheses:

H1: 80% or more of sites will have earthworms present and/or be in an impacted state.

H2: Graminoid abundance will be associated with *Lumbricus rubellus* and *Lumbricus terrestris* presence

H3: Browsing should be extensive across the landscape, and variation in browsing intensity should be related to graminoid occurrence. Occurrence of palatable or preferred species and abundance of tree regeneration attaining heights of one meter or more should be reduced.

Hypotheses H1:

The mean earthworm present site occurrence for the two non-drought years was 90% of randomly selected sites, above the Holdsworth et al. estimate of 80% of the landscape as in an earthworm impacted state (2007b). The more severe impact categories three and four were the most populated categories, indicating that earthworms have been present for some time. Additionally, occurrence is spatially extensive throughout both forest units (Maps Appendix C).

Hypothesis 2: The species was strongly related to *Lumbricus rubellus* presence by Ordination and Indicator Species Analysis. Multiple Response Permutation Procedure indicated the two communities were highly divergent; species richness estimation showed the Acer Saccharum sites with greater mean cover, diversity and estimated diversity. Additionally, the Invasion Effect classes 3 and 4 (which are characterized by *Lumbricus* presence) were the most frequent. However, *Carex pensylvanica*'s relationship to *Lumbricus terrestris* does not appear clear in this study.

Hypothesis 3:

Due to extensive heavy browsing and lack of lightly browsed sites for comparison, browsing effect was untestable within this study. However, the results do concur with studies from the same region for increased graminoid cover related to deer browsing (Wiegmann and Waller 2006; Rooney 2009).

The individual responses appear to justify the following arguments:

1: The forest understory currently has two dominant and divergent states: One graminoid dominated, driven by both earthworms and deer browsing, and, the remaining *Acer saccharum* dominated sites. Ordination, Indicator Species Analysis and Multiple Response Permutation Procedure indicate two distinct communities with earthworms and deer browsing as probable causes for Carex pensylvanica cover dominance.

2: *Carex pensylvanica* and *Lumbricus rubellus* appear to strongly co-occur.

3: Non-pigmented (anecic) earthworm taxa appear to also co-occur strongly with *Carex pensylvanica*, but not as strongly as *Lumbricus rubellus*. This concurs with Frelich Earthworm Invasion Effect Category 3.

General Discussion: Earthworms

The evidence regarding earthworm invasion altering vegetative communities in North America is well documented. The mechanisms are well described; earthworms, depending on species and/or species assemblage, first affect decomposition processes by altering and quickening litter decomposition. Litter biomass has been found negatively correlated with earthworm biomass in multiple studies (Suarez et al. 2006; Nuzzo et al. 2008; Eisenhauer et al. 2007). The processing rate varies with litter species (Holdsworth et al. 2012; Hendriksen 1990) and earthworm species and species assemblages (Hale et al. 2005; Gundale 2002). Sugar maple (*Acer saccharum*) and American basswood (*Tilia Americana*) leaf litter are among the favored litter species for earthworms, especially *Lumbricus* species. They are also among the most common trees on sites included in this study, and thus high levels of earthworm impacts are expected. Earthworms, depending upon the species or species assemblage, process litter and mix the soil horizons mechanically. Soil structure is changed from well defined “O” (organic) and “A” (mineral) horizons to “mull humus” (Bohlen et al. 2004), and in certain cases with high earthworm biomass, bare soil surface (Hale 2006). Litter biomass has been found negatively correlated with earthworm biomass in multiple studies (Suarez et al. 2006; Nuzzo et al. 2008; Eisenhauer et al. 2007). Reduction or elimination of the litter layer and the consequent "O" horizon alters or reduces multiple components of the litter community including mychorrhizal fungi (Fisk et al. 2002), soil microbe biomass (Groffman et al. 2004; Eisenhauer et al. 2011) and litter dwelling species and invertebrates (Migge-Kleian 2006). Earthworm-invaded sites also show reduced or altered fine root mass, especially in *Lumbricus terrestris* invaded sites but below 10 cm depth (Fisk et al 2004, Hale et al. 2005b). Soil physical properties are altered; organic material is trans-located downward to the A horizon with increased bulk density and horizon thickness (Bohlen et al. 2004b; Hale et al. 2005b), and these changes in soil structure lead to drying of soil surface layers. Soil nutrient locations and flows are altered with reduced total soil carbon, soil C and N transferred and stored at lower soil levels (Wironen and Moore 2006), and lower availability of N and P in surface soil layers (Hale et al. 2005b). Changes in water and nutrient cycling, as well as seedbed conditions (litter versus mineral soil) and changes in soil microflora and fauna can have large impacts on the plant community (Hale et al. 2006; Holdsworth et al. 2007; Eisenhauer et al. 2009; Eisenhauer et al. 2012). Species with traits suitable for this new environment include reproductive strategies such as vegetative reproduction or seeds able to cope with thin organic horizons (Hale et al. 2006; Holdsworth et al. 2007; Eisenhauer et al. 2009). Particular to this study is *Carex pensylvanica* with useful traits for the new environment; vegetative reproduction through rhizomes, early season seed dispersal, adaptation to browsing (silica content and secondary compounds), ability to withstand drought and growth habit (low protected meristems) (Coughenour 1981; Rooney 2009; Wiegmann and Waller 2006).

Relationship to Previous Studies

The results concur to regional studies both within the Chequamegon-Nicolet national Forest and the western Great Lakes Region. Holdsworth et al. (2007a), in a study that included the Chequamegon, found that there was an 81% probability a site contained a *Lumbricus* species - *Aporrectodea* community assemblage within 500 meters of a lake and a significant relationship between reduced plant species richness and *Lumbricus* species biomass. There were similar results for *Carex pensylvanica* in heavily invaded sites, both in ordination and indicator species tests with an indicator species result of 72 for *Carex pensylvanica* in heavily invaded Chequamegon plots (Holdsworth 2007a; 2007b). Fisichelli et al. (2013), within the western Great Lakes including both of these forests, found a slightly lower proportion of the landscape with high severity of earthworm invasion (49% of sites), however, his study included more remote areas such as Upper Michigan and northern Minnesota where earthworm invasion is likely not as advanced as in Wisconsin due to later dates of European settlement. Hale et al. (2006), found reduced abundance and species richness in herbaceous community structure related to earthworm biomass. Earthworm community composition had significant impacts to invasion severity, with a relationship between *Carex pensylvanica* and *Lumbricus rubellus* in NMDS ordination.

General Discussion: White Tailed Deer Browsing

While *Odocoileus virginianus* is native to the region, its population has expanded both in numbers and range in the last century (Cote et al.2004). Whitetail deer browsing is linked to increased graminoid presence (Rooney and Waller 2003, Powers and Nagel 2008), reduced wildflower populations (Hale et al. 2007, Rooney and Gross 2003) and reduced diversity and regeneration of selected canopy taxa, including conifers (Jordan 1967, Cornett et.al. 2000, Allison 1991). These vegetative community changes occur throughout different landscapes. Studies have found impacts in near - boreal conditions in Canada (Coté et al. 2004), mesic hardwood forests in northern Wisconsin (Rooney 2009, Wiegmann and Waller 2006) and fragmented woodlots in Southern Minnesota (Augustine & Frelich 1998). Winter browsing has been linked to reduced hemlock and cedar recruitment (Frelich and Lorimer 1991, Rooney at al. 2001) and, on sites where more desirable seedlings are not present, reduced *Acer saccharum* regeneration (Wiegmann and Waller 2006, Salk et al. 2011). Through selective summer browsing, diversity is reduced from dominance by forbs to graminoids (Wiegmann and Waller 2006, Coté et al. 2004, Rooney 2009).

Regional white-tailed deer browsing studies have similar results; heavy white-tailed deer browsing is common across the landscape with the understory shifting towards graminoid dominance. Wiegmann and Waller (2006) resurveyed sites originally reported in Curtis (1959); Graminoid species increased across the study area, *Poaceae* (54%) and *Carex* species (286%) respectively. Rooney et al. (2004), using the same site database for resurvey, found overall lower species richness, loss of native species, increased graminoid cover and “biotic impoverishment”(Curtis 1959). Rooney (2009), in an exclosure study from the same landscape region as this work, found deer herbivory to have shifted the understory community to one graminoid dominated; sedges were the dominant understory cover type outside exclosures.

There are complex relationships, however; Fisichelli et al. (2013) found deer herbivory linked to temperature (higher temperatures increased deer browsing) and worm presence structured by soil pH, precipitation and initial community (conifer communities having less original earthworm presence). Holmes and Webster (2010) found disturbance gap size, in this case experimentally designed removal of hardwood species, affected deer herbivory and post-disturbance vegetative community structure. The previously mentioned study by Powers and Nagel (2008) determined that three interacting factors, intensive silvicultural management, high earthworm density and heavy deer browsing could contribute to *Carex pensylvanica* cover dominance.

Summary

This study's results indicate the forest understory is heavily browsed and impacted by earthworms across broad areas of both forest units. If the sites accurately represent the rest of the forest, large areas of the herbaceous understory are now dominated by graminoids, especially *Carex pensylvanica*, with fewer forbs and reduced overall diversity. There appears to be reduced seedling recruitment of canopy species, though the lack of lightly browsed sites in this study limits comparison. That, however, is important unto itself as these sites were randomly selected and are broadly spaced; browsing appears heavy across the entire landscape of both forests. At the shrub and sapling layer, both shrub and potential canopy species appear to have reduced recruitment. The heavily impacted categories "3" and "4" are the most frequently occurring, concurring with this other findings. These vegetative community results are similar to other studies from the same region and it is likely the same changes are occurring outside of the forests within the region. *Lumbricus rubellus* is well established across a broad range of both forest units and is strongly linked to *Carex pensylvanica* occurrence by Ordination, Multiple Response Permutation Procedure and Indicator Species Analysis. The results for relationships between Lumbricus terrestris and other variables are limited. The species does occur in fifty percent of sites in the two non-drought years, indicating they are established in broad areas of the forest. It appears they are not as strongly linked to *Carex pensylvanica* presence as *Lumbricus rubellus*; however, other variables such as soils, time of invasion, hydrology and other factors may have affected results. The remaining *Acer saccharum* community sites, affected by browsing and earthworms, will likely move towards the Graminoid dominated state in the future as neither earthworm nor white-tail deer populations respect property boundaries and are limited only by landscape features (Gundale 2005; Holdsworth et al. 2007a). Remaining *Acer saccharum* community sites likely require protection and adaptive management by limiting new earthworm introduction pathways (mostly human access and behavior) (Holdsworth et al. 2007a) and minimizing deer herbivory by protection, increased hunting and/or greater predation. Most likely, management interventions to increase canopy species recruitment will be needed in the future, whether silvicultural, protective or by wildlife population management. Interventions would need to be multi-faceted, combining multiple techniques over time to increase recruitment or improve habitat. This study did not consider climate warming and both temperature and precipitation changes will impact community composition. The easiest, cheapest yet most socially difficult management tool would be to increase the population of the most able carnivore, the Eastern Timberwolf. A significant reduction in Whitetail deer population would likely have the most impact for the vegetative community. The forest would be different in composition, but there would be a forest. At the current recruitment rates, I do question the type and quantity of forest that will exist in just a few decades.

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Appendices

Appendix A: Data Sheet

Data Sheet

Date:____________ Time:____________

Weather:____

Stand ID#_____________________
Landscape Description:____
Slope degrees, aspect, degrees_______________________________________

Subplot herbaceous, shrub and tree seedling vegetation <1 m, by coverclass

Subplot 1		Subplot 2		Subplot 3		Subplot 4	
Taxa	Cover	Taxa	Cover	Taxa	Cover	Taxa	Cover

Subplot shrubs >1 m tall, by coverclass

Subplot 1		Subplot 2		Subplot 3		Subplot 4	
Taxa	Cover	Taxa	Cover	Taxa	Cover	Taxa	Cover

Subplot tree saplings 1-2 m tall, by density class

Subplot 1		Subplot 2		Subplot 3		Subplot 4	
Taxa	density	Taxa	density	Taxa	density	Taxa	density

Subplot tree saplings >2 m to 2.5 cm dbh, by density

Subplot 1		Subplot 2		Subplot 3		Subplot 4	
Taxa	density	Taxa	density	Taxa	density	Taxa	density

Plot-wide data (30 x 30 m):

Basal area 10baf from center — Additional taxa (trees, shrubs, herbs)

Taxa	dbh		Taxa	Abundance category

Browsing: Woody____________________ Herbaceous_____________________
Worm samples 1 and 2 taken: sample 1:_____________ sample 2____________
Forest floor description, (L, F and H thickness):

Layer	Sample 1	Sample 2
L		
F		
H		

Soil sample taken for texture analysis:______

Plot
Comments:____

Appendix B: Worm Data Sheet:

Worm Data Sheet

Stand ID#_________________

Observer:_____________ Date:________________ Time:_________________

Weather:____

Groundcover Description:____

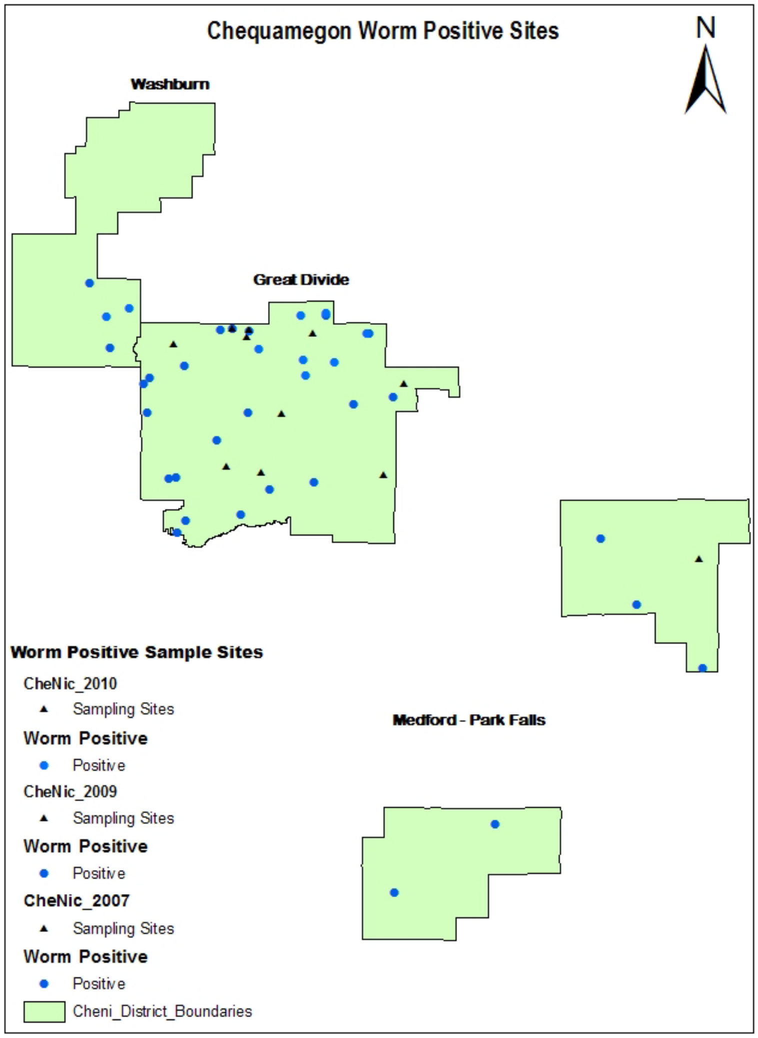
Middens Observed:

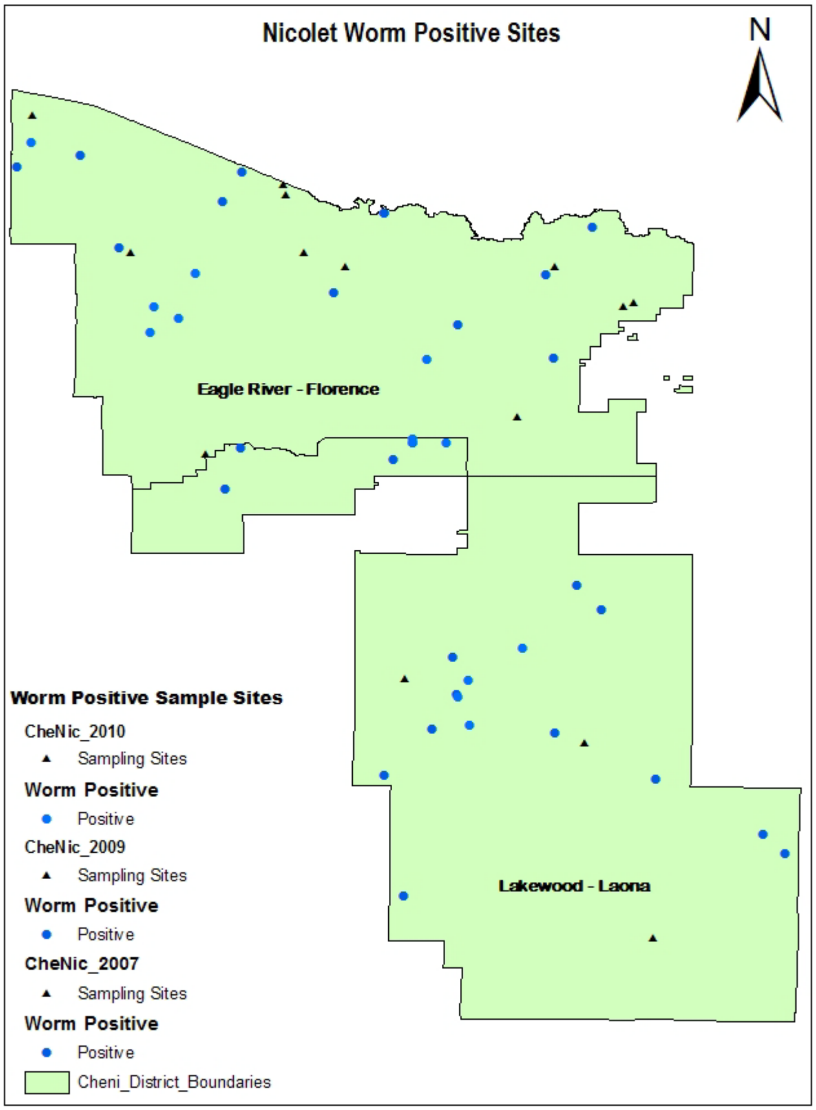
Taxa Observed and/or Extracted:

Vials Filled:

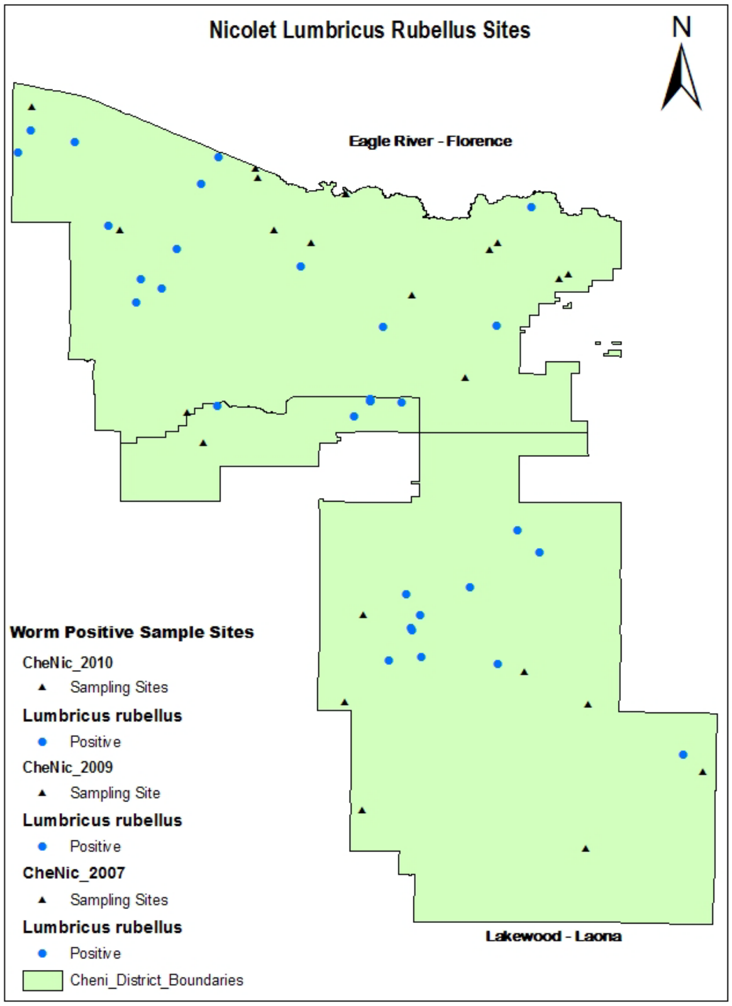
Make Sure to ID vials by Stand, Date and Time.

Appendix C: Maps

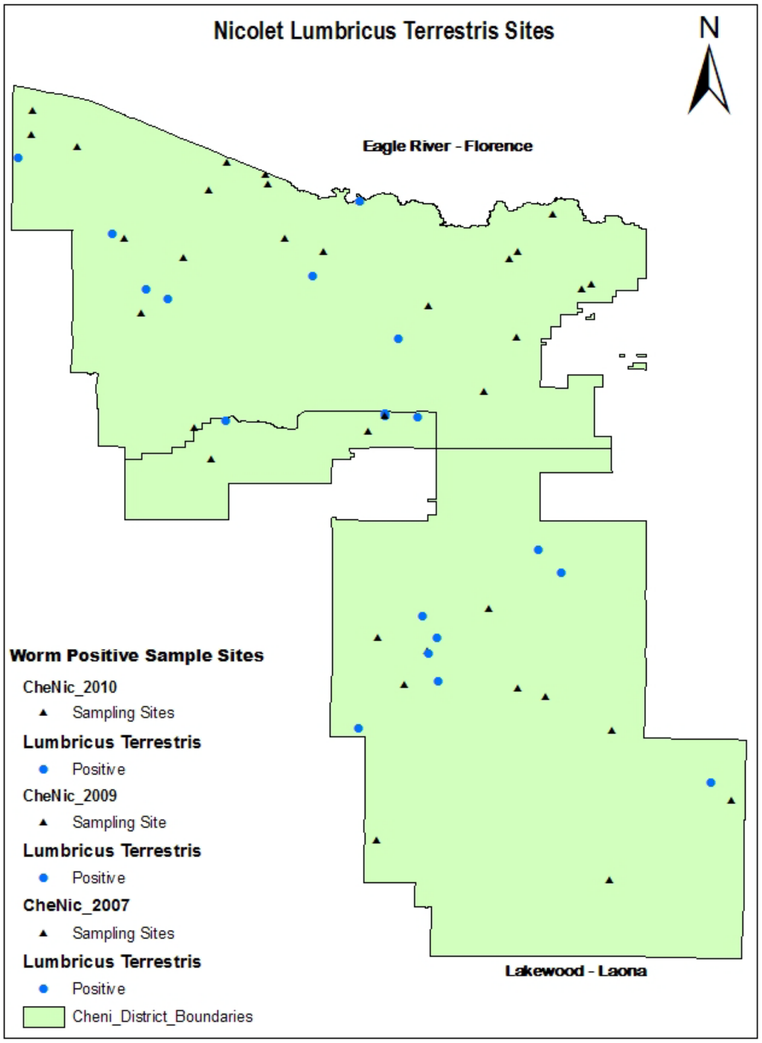

Chequamegon Worm Positive Sites
N
Washburn
Great Divide
Medford - Park Falls
Worm Positive Sample Sites
CheNic_2010
Sampling Sites
Worm Positive
Positive
CheNic_2009
Sampling Sites
Worm Positive
Positive
CheNic_2007
Sampling Sites
Worm Positive
Positive
Cheni_District_Boundaries



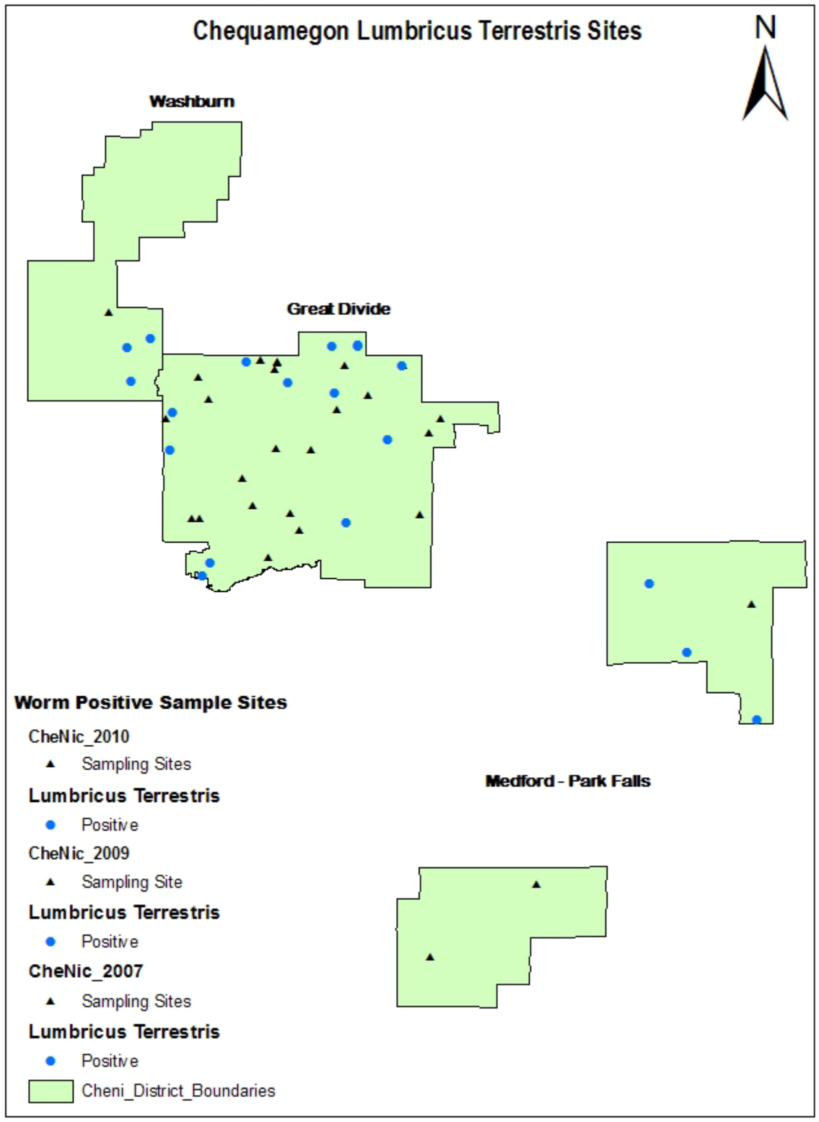
Map 4: 2007, 2009-2010 data. Colored circles are earthworm positive sites regardless of species. Triangles are earthworm free sites.



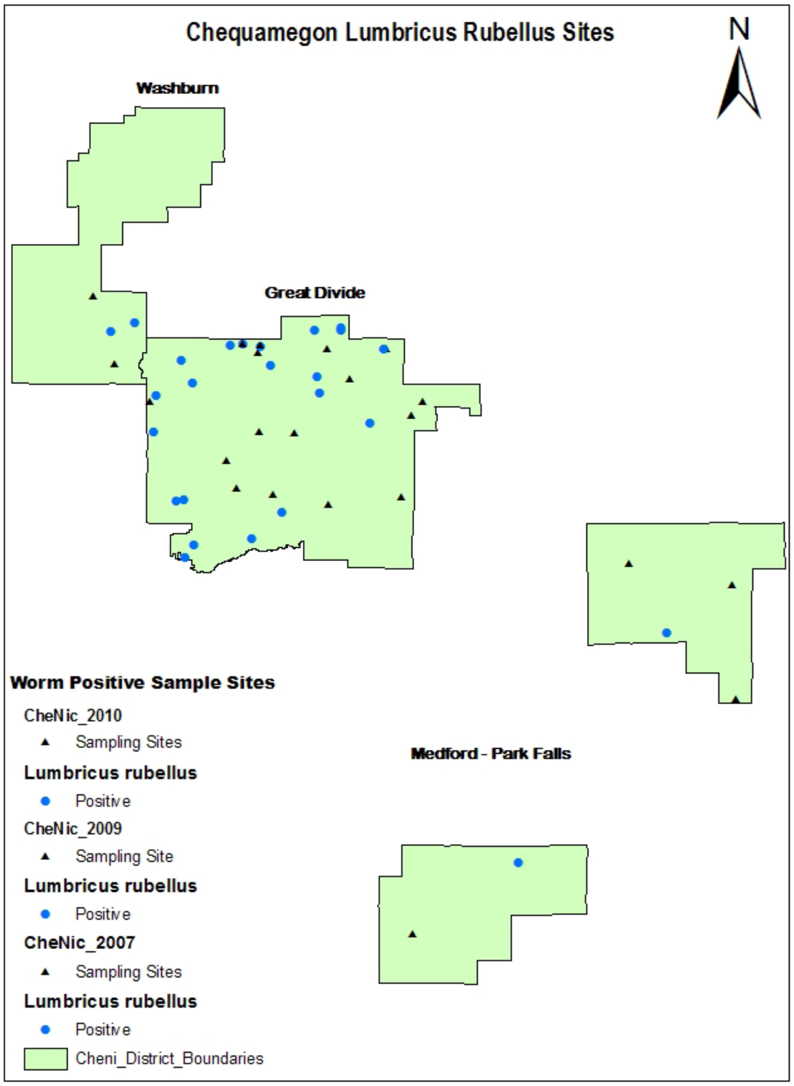
Map 5: Nicolet *Lumbricus rubellus* positive sites.2007, 2009-2010 data. Circles are *Lumbricus rubellus* positive sites. Triangles are *Lumbricus rubellus* free sites.



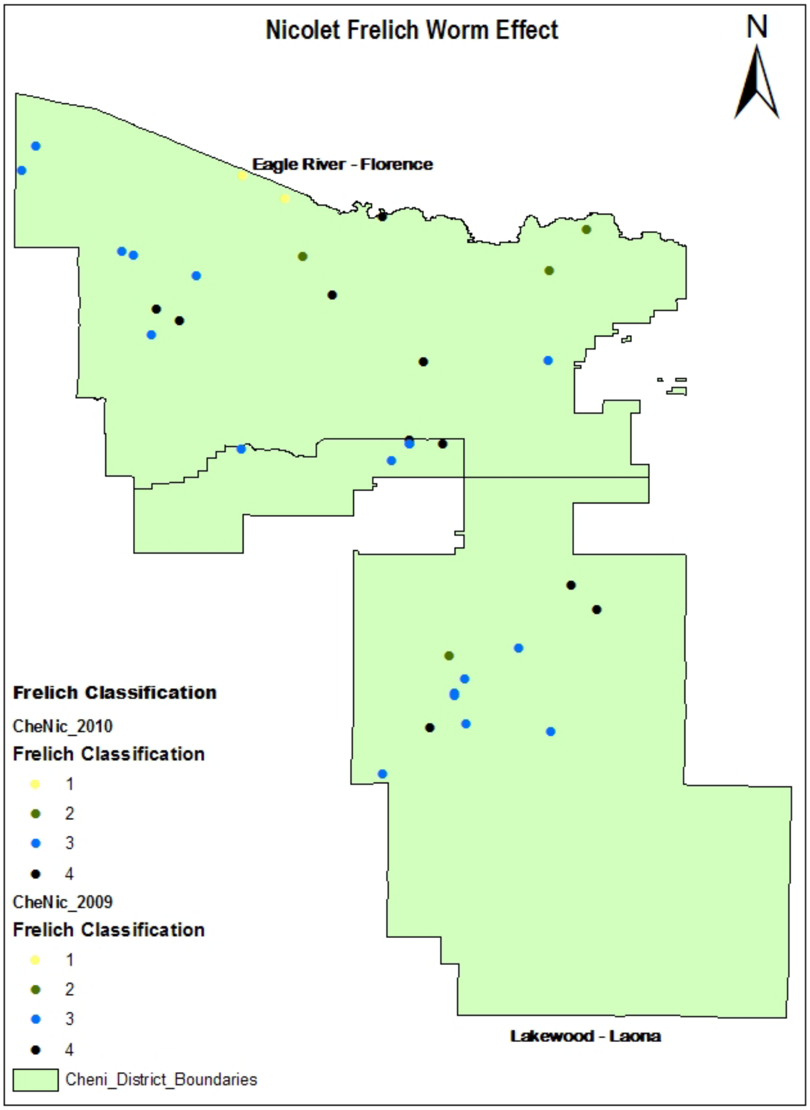
Map 7: Nicolet *Lumbricus terrestris* positive sites. 2007. 209-2010 data. Circles are *Lumbricus terrestris* positive sites. Triangles are *Lumbricus terrestris* free sites.



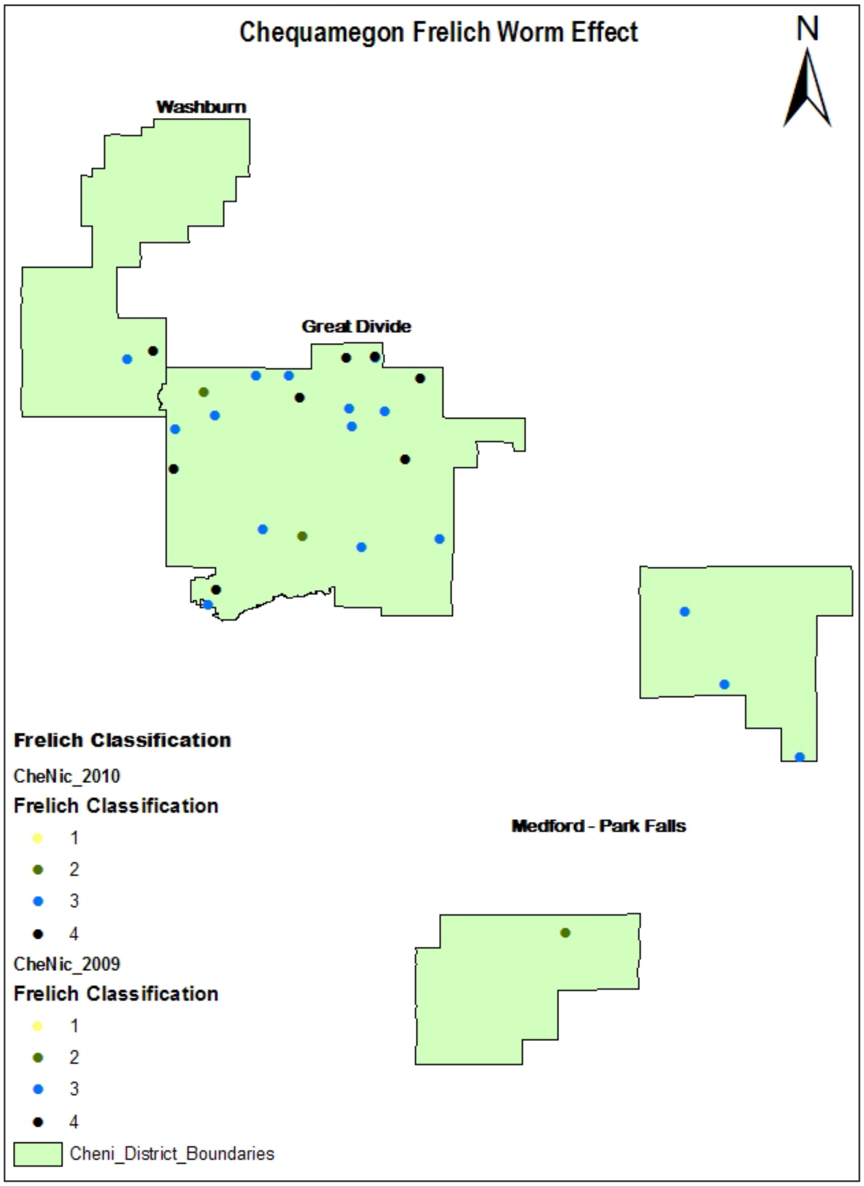
Map 8: Chequamegon *Lumbricus terrestris* positive sites. 2007, 2009-2010 data. Circles are *Lumbricus terrestris* positive sites. Triangles are *Lumbricus terrestris* free sites.



Map 6: Nicolet *Lumbricus rubellus* positive sites.2007, 2009-2010 data. Circles are *Lumbricus rubellus* positive sites. Triangles are *Lumbricus rubellus* free sites.



Map 9: 2009-2010 data. Earthworm Invasion Categories Following Frelich, 2009.



Map 10: Earthworm Invasion Categories Following Frelich, 2009-2010 data.

Appendix D: Species/Taxa – Plant Symbol List

Carex pensylvanica	CAPE6
Acer saccharum	ACSAS
Trientalis borealis	TRBOB
Aralia nudicaulis	ARNU2
Maianthemum canadense	MACA4
Coptis trifolia	COTR2
Lycopodium complanatum	LYCO3
Polygonatum biflorum	POBI2
Poaceae sp	POAUN
Trillium sp	TRISP
Dryopteris sp	DRYOSP
Osmorhiza longistylis	OSLO
Trillium cernuum	TRCE
Rubus idaeus	RUID
Adiantum pedatum	ADPE
Maianthemum racemosum	MARAR
Brachyelytrum erectum	BRER2
Dryopteris intermedia	DRIN5
Carex sp	CARUN
Dirca palustris	DIPA9
Allium tricoccum	ALTR3
Arisaema triphyllum	ARTR
Trillium grandiflorium	TRGR4
Carex plantaginea	CAPL4
Eurybia macrophylla	EUMA27
Caulophyllum thalictroides	CATH2
Hepatica acutiloba	HENOA
Polygonatum pubescens	POPU4
Corylus cornuta	COCO6
Pteridophyte unid (Fern)	FERNUN
Hepatica nobilis var obtusa	HENOO
Boehmeria cylindrica	BOCY
Populus tremula	POTR5
Polypodiaceae unid	POLYPOD
Amelenchier sp	AMEL
Osmunda cinnamomea	OSCI
Viola canadensis	VICA4
Galium asprellum	GAAS2
Viola pubescens	VIPUP2
Solidago flexicaulis	SOFL2
Ostyra virginiana	OSVI
Ranunculus recurvatus	RARE2
Pyrola elliptica	PYEL
Betula papyrifera	BEPAP
Lonicera canadensis	LOCA7

Linnaea borealis	LIBOA
Abies balsamea	ABBAB
Cornus canadensis	COCA13
Sanguinaria canadensis	SACA13
Lycopodium dendroideum	LYDE
Clintonia borealis	CLBO3
Lycopodium clavatum	LYCL
Dryopteris expansa	DREX2
Carex oligosperma	CAOL3
Diervilla lonicera	DILO
Fraxinus americana	FRAM2
Ribes cynosbati	RICY
Vaccinium angustifolium	VAAN
Asarum canadense	ASCA
Oryzopsis asperifolia	ORAS
Galeopsis tetrahit	GATE2
Galium triflorum	GATR3
Viola blanda	VIBL
Sonchus oleraceus	SOOL
Pilosella aurantiaca	HIAU
Actaea pachypoda	ACPA
Carex oligocarpa	CAOL3c
Anenome virginiana	ANVI3
Carex radiata	CARA8
Rubus pubescens	RUPUP2
Thalictrum dioicum	THDI
Bryophyte unid	BRYOUN
Plantago major	PLMA2
Tilia americana	TIAMA
Betula alleghaniensis	BEALA
Fraxinus sp	FRAXSP
Sambucus nigra	SANIC4
Convolvulus sp	Convspp
Fragaria vesca	FRVE
Potentilla canadensis	POCA17
Centaurea sp	Centspp
Gaultheria procumbens	GAPR2
Chimaphila umbellata	CHUM
Quercus rubra	QURU
Pinus resinosa	PIRE
Viburnum acerfolium	VIAC
Cornus sericea	COSE16
Amelenchier sp	Amelspp
Carex pedunculata	CAPE4
Tsuga canadensis	TSCA

Pteridium aquilinum	PTAQ
Rubus flagellaris	RUFL
Panax trifolius	PATR2
Oxalis montana	OXMO
Vaccinium myrtilloides	VAMY
Fagus grandifolia	FAGR
Urtica dioica	URDI
Symphyotrichum puniceum	SYPU
Acer spicatum	ACSP2
Rubus parviflorus	RUPA
Carex peduncualata	CAPE4
Hepatica nobilis	HENO2
Asteraceae sp	ASTERSP
Lycopodium obscurum	LYDE
Anemonella thalictroides	THTH2
Carpinus caroliniana	CACA18
Panax quinquefolium	PAQU
Huperzia lucidula	HULU2
Taenidia integerrima	TAIN
Pinus strobus	PIST
Anemone quinquefolia	ANQU
Gymnocarpium dryopteris	GYDR
Anemone canadensis	ANCA8
Streptopus lanceolatus	STLAC
Gaultheria hispidula	GAHI2
Gymnocarpium intermedia	GYMINT
Veronica officinalis	VEOFO
Lycopodium complanatum	LYCO3
Athyrium filix-femina	ATFI
Osmunda claytoniana	OSCL2
Salix sp	SALSPP
Sonchus sp	SONCH
Pyrola secunda	ORSE
Dryopteris cristata	DRCR4
Onoclea sensibilis	ONSE
Hieracium vulgatum	HILA8
Monotropa uniflora	MOUN3
Impatiens capsensis	IMPA
Carya ovata	CAOV2
Uvularia sessilifolia	UVSE
Taraxacum officinale	TAOF

Appendix E: Field Research Protocol

Field Research Protocol:

1) Using GPS coordinates and other navigation locate to SW corner of research plot
2) Locate 30 x 30 m plot in relatively uniform landscape and flag (avoid breaks within plot, swamps, water,bedrock,etc)
3) Assign 4 2m Vegetation sampling subplots nested at 10 m grid points within main plot
4) Assign 2 worm sampling points on vertical axis (N-S) grid points between vegetation subplots.
5) Perform Worm Sampling Protocol
6) Classify plants in subplots to 1 m tall in one of six classes by dominant taxa including herbaceous vegetation, tree seedlings and others all in % cover classes as assigned according to the following<1%= Class1,1-5=Class 2,5-25 = Class3,25-50=Class4,50-75=Class 5,75-100 =Class 6
7) Record Percentage cover of Tree saplings 1- 2 m tall not greater than 2.5 cm dbh
8) On 4 plots observe and record density saplings, greater than 1 M tall, no greater than 2.5 cm dbh
9) Record in density classes per taxa, 1-5=1; 5-10=2; 10-20=3; 20-30=4
10) Observe tall shrubs over 1 meter throughout fullplot ,classify by % cover
11) Classify trees from center of plot , calculating basal area from dbh using a ten basal area factor prism
12) Classify browsing intensity, sugar maple saplings between 1 to five feet estimate % browsed
13) Hike border of big plot and list all taxa not present on subplots by low, medium and high abundance.
14) Cut a 15 x 15 cm square on each four subplots of forest floor and put in a paper bag; take soil sample from underneath creating composite sample for plot, placing soil sample in whirlpak.

Appendix F:

Dominance Curve Test Results:

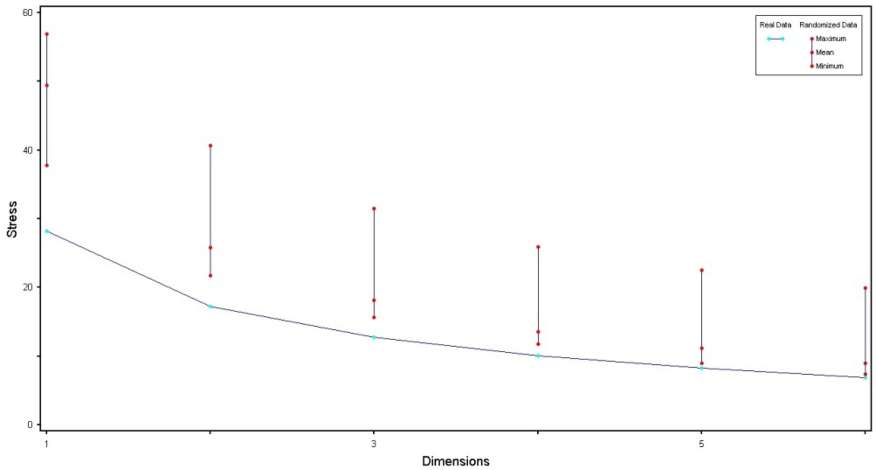


Figure 3: Plot of Stress vs. Dimensions: NMDS Fifteen Percent Taxa Removed: Real data stress is below randomization test.

Cover Pe	RankAbun	Log(SumAbund)	Sum	RankFreq	Freq	Mean	S.Dev.	V/M	
ACSAS	2	1.34412	22.0859	2	49	0.356224	0.377294	105.9149	0.3996
CAPE6	1	1.69086	49.0745	1	58	0.791524	0.333706	42.1600	0.1407
CARUN	4	1.12252	13.2592	3	43	0.213858	0.277254	129.6438	0.3594
DRIN5	6	0.952543	8.96484	6	30	0.144594	0.245290	169.6403	0.4161
DRYOSP	9	0.787596	6.13191	8	21	0.989018E-01	0.215709	218.1037	0.4705
FRAXSP	10	0.736559	5.45204	11	18	0.879361E-01	0.206390	234.7048	0.4844
MACA4	3	1.12753	13.4132	4	43	0.216342	0.263321	121.7150	0.3205
OSVI	12	0.513018	3.25851	12	18	0.525565E-01	0.114099	217.0979	0.2477
POAUN	11	0.661790	4.58976	9	21	0.740284E-01	0.171559	231.7480	0.3976
POPU4	7	0.869337	7.40179	7	25	0.119384	0.217015	181.7794	0.3945
TRBOB	5	0.995984	9.90796	5	33	0.159806	0.275721	172.5353	0.4757
TRISP	8	0.827236	6.71793	10	21	0.108354	0.221800	204.7002	0.4540

Appendix F: Sampling Power Test Results

Kendall's Tau Test Power Analysis using Simulation

Simulation Summary

Variables X and Y	Power (H1) Simulation Distribution	Alpha (H0) Simulation Distribution
	Bivariate Normal	Bivariate Normal

Random Number Pool Size	1000000
Number of Simulations	500000

Numeric Results for Testing Correlation Hypotheses: H0: $\tau = 0$; H1: $\tau \neq 0$

Row	Sample Size N	Target Power	Actual Power	H0 Corr $\rho0$	H1 Corr $\rho1$	Target Alpha	Actual Alpha
1	43	0.900	0.906	0.000	0.500	0.050	0.048

Run Time: 16.60 minutes.

References

Kendall, M. and Gibbons, J.D. 1990. Rank Correlation Methods, 5th Edition. Oxford University Press. New York.
Devroye, Luc. 1986. Non-Uniform Random Variate Generation. Springer-Verlag. New York.

Report Definitions

N is the size of the sample drawn from the population. It is the number of X-Y data points in a sample.
Target Power is the power that was desired. A search was made to find the smallest sample size that would
achieve this power.
$\rho0$ is the Pearson correlation coefficient assuming the null hypothesis, H0, which is set to zero which results
in a test of non-correlation between X and Y.
$\rho1$ is the Pearson correlation coefficient assuming the alternative hypothesis, H1. This is the value at which
the power is computed.
Target Alpha is the probability of rejecting a true null hypothesis. It is set by the user.
Actual Alpha is the alpha level that was actually achieved by the experiment. It is calculated by the alpha
simulation.
Beta is the probability of accepting a false null hypothesis.

Summary Statements

A sample size of 43 achieves 91% power to detect a Pearson correlation of 0.500 using a two-sided hypothesis test with a significance level of 0.050. These results are based on 500000
Monte Carlo samples from the bivariate normal distribution under the alternative hypothesis.

Power and Alpha Confidence Intervals from Simulations

Row	Sample Size N	Target Power	Actual Power	Lower Limit of 95% C.I. of Power	Upper Limit of 95% C.I. of Power	Target Alpha	Actual Alpha	Lower Limit of 95% C.I. of Alpha	Upper Limit of 95% C.I. of Alpha
1	43	0.900	0.906	0.905	0.907	0.050	0.048	0.047	0.049

Definitions of the Power and Alpha Confidence Intervals Report
N is the size of the sample drawn from the population. It is the number of X-Y data points in a sample.
Target Power is the planned probability of rejecting H0 when it is false.
Actual Power is the probability of rejecting H0 when it is false. This is the actual value calculated by the
power simulation.
Lower and Upper Limits of a 95% C.I. for Power are the limits of an exact, 95% confidence interval for power
based on the binomial distribution. They are calculated from the power simulation.
Target Alpha is the desired probability of rejecting a true null hypothesis at which the tests were run.
Actual Alpha is the alpha achieved by the test as calculated by the alpha simulation.
Lower and Upper Limits of a 95% C.I. for Alpha are the limits of an exact, 95% confidence interval for alpha
based on the binomial distribution. They are calculated from the alpha simulation

Kendall's Tau Test Power Analysis using Simulation

Simulation Summary

Variables	Power (H1) Simulation Distribution	Alpha (H0) Simulation Distribution
X and Y	Bivariate Normal	Bivariate Normal

Random Number Pool Size	1000000
Number of Simulations	500000

Numeric Results for Testing Correlation Hypotheses: H0: $\tau = 0$; H1: $\tau \neq 0$

Row	Sample Size N	Power	H0 Corr $\rho 0$	H1 Corr $\rho 1$	Target Alpha	Actual Alpha
1	62	0.978	0.000	0.500	0.050	0.049

Run Time: 2.78 minutes.

References
Kendall, M. and Gibbons, J.D. 1990. Rank Correlation Methods, 5th Edition. Oxford University Press. New York.
Devroye, Luc. 1986. Non-Uniform Random Variate Generation. Springer-Verlag. New York.

Report Definitions
N is the size of the sample drawn from the population. It is the number of X-Y data points in a sample.
Power is the probability of rejecting a false null hypothesis. It is calculated by the power simulation.
$\rho 0$ is the Pearson correlation coefficient assuming the null hypothesis, H0, which is set to zero which results
in a test of non-correlation between X and Y.
$\rho 1$ is the Pearson correlation coefficient assuming the alternative hypothesis, H1. This is the value at which
the power is computed.
Target Alpha is the probability of rejecting a true null hypothesis. It is set by the user.
Actual Alpha is the alpha level that was actually achieved by the experiment. It is calculated by the alpha simulation.
Beta is the probability of accepting a false null hypothesis.

Kendall's Tau Test Power Analysis using Simulation

Summary Statements
A sample size of 62 achieves 98% power to detect a Pearson correlation of 0.500 using a two-sided hypothesis test with a significance level of 0.050. These results are based on 500000
Monte Carlo samples from the bivariate normal distribution under the alternative hypothesis.

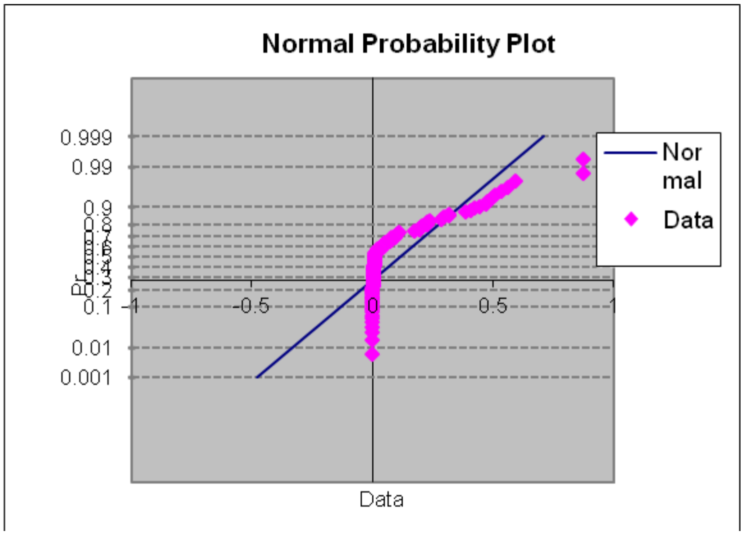
Power and Alpha Confidence Intervals from Simulations

Row	Sample Size N	Power	Lower Limit of 95% C.I. of Power	Upper Limit of 95% C.I. of Power	Target Alpha	Actual Alpha	Lower Limit of 95% C.I. of Alpha	Upper Limit of 95% C.I. of Alpha
1	62	0.978	0.978	0.978	0.050	0.049	0.049	0.050

Definitions of the Power and Alpha Confidence Intervals Report
N is the size of the sample drawn from the population. It is the number of X-Y data points in a sample.
Power is the probability of rejecting H0 when it is false. This is the actual value calculated by the power
simulation.
Lower and Upper Limits of a 95% C.I. for Power are the limits of an exact, 95% confidence interval for powerbased on the binomial distribution. They are calculated from the power simulation.
Target Alpha is the desired probability of rejecting a true null hypothesis at which the tests were run.Actual Alpha is the alpha achieved by the test as calculated by the alpha simulation.Lower and Upper Limits of a 95% C.I. for Alpha are the limits of an exact, 95% confidence interval for alpha based on the binomial distribution. They are calculated from the alpha simulation

Appendix G: Effect size calculation
Mann-whitney test:
Z-Score is =4.1353. p-value is 0. result is significant at $p \leq 0.05$.
U-value = 1094. Distribution is approximately normal since the n= or >10 . Therefore, the Z-value can be used effect= r= z/square root of N= 0.371
For the wilcoxon signed rank the effect ends up as 0.414 effect is .400

Appendix H: Anderson-Darling Normality Test:


Normal Probability Plot
0.999
0.99
0.9
0.1
0.01
0.001
-1
-0.5
0
0.5
1
Data
Normal
Data